Science Library

APR 18 1950

Volume 40 *Number 4*

133

Phytopathology

AN INTERNATIONAL JOURNAL

April 1950

Official Organ of

The American Phytopathological Society

$7.75 per year, Canada · $7.50 per year, United States and dependencies $8.00 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter. Acceptance for mailing at the special rate of postage provided for in paragraph (d-2) Section 34.40, P. L. & R. of 1948. Authorized May 12, 1921.

TABLE OF CONTENTS

The wilt disease of American persimmon, caused by Cephalosporium diospyri ..... Bowen S. Crandall and W. L. Baker 307

Relation of concentration of some organic substances to spore germination and dosage response ..... Harold J. Miller 326

Effectiveness of fungicidal sprays and dusts in azalea petal blight control ..... D. L. Gill 333

Physiology of toxin production by Ceratostomella ulmi. A. W. Feldman, N. E. Caroselli, and F. L. Howard 341

Root and collar winter injury of apple trees. J. S. Cooley and A. B. Groves 355

Studies on safflower rust in India. Raghubir Prasada and Homi P. Chothia 363

Vertical distribution of Verticillium albo-atrum in soils. Stephen Wilhelm 368

Some interrelationships of virus strains in sugar-beet curly top. N. J. Giddings 377

Phytopathological Notes:

A modification of the van Tiegham cell for purification of contaminated fungus cultures. P. A. Ark and Robert S. Dickey 389

A technique for observing tuber enlargement and scab development in potatoes ..... W. J. Hooker 390

Natural occurrence of tobacco necrosis virus in primrose. W. C. Price, Frank P. McWhorter, and Betty H. Steranka 391

The effect of yellow dwarf on yield of onion seed. R. D. Watson and Glenn KenKnight 392

Report of the 41st Annual Meeting of the American Phytopathological Society 394

Minutes of the Northeastern Division, The American Phytopathological Society, December 28–30, 1949 406

THE WILT DISEASE OF AMERICAN PERSIMMON, CAUSED BY CEPHALOSPORIUM DIOSPYRI

BOWEN S. CRANDALL[1] AND W. L. BAKER[2]

(Accepted for publication July 15, 1949)

In 1936 a report was made to the U. S. Department of Agriculture that persimmon trees, *Diospyros virginiana* L., were dying southeast of Nashville, Tennessee. Examination revealed that the trees were being killed by a vascular wilt disease. The pathogen causing this disease was found to be an undescribed species of fungus of the genus *Cephalosporium*, subsequently described and named *C. diospyri* Crandall (1).

A preliminary investigation of the disease by the Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, was made in the spring of 1937. Field studies of the disease were initiated in the spring of 1938 by the Division of Forest Pathology, and by the Division of Forest Insect Investigations of the Bureau of Entomology and Plant Quarantine, to determine the means of entry into trees, and how the disease spread. Additional studies were made by the senior author to test the pathogenicity of the fungus, its host range, and development in the host. Observations on the geographical range of the disease were made in 1938 and 1939 and were repeated in part on a somewhat limited basis in 1943 and 1948.

The common persimmon, *Diospyros virginiana*, occurs throughout the eastern and east-central States, from central Iowa, northern Illinois, Indiana, Ohio, southern Pennsylvania, and New Jersey to the Gulf of Mexico and to Lake Okeechobee in Florida. The tree is important as a source of food for wildlife and to some extent for man and domesticated animals. From a lumber standpoint its use is specialized and limited. The heavy, white, easily worked wood makes it ideally suited for golf club heads. Its hardness and ability to take a high polish also make it suitable for shuttles. In some regions the tree is important in soil conservation, being one of the first, or often the first, tree species to start growth on abandoned and denuded land.

SYMPTOMS

External symptoms of the disease are typical of vascular infection. The symptom most commonly encountered is an abrupt wilting of the foliage (Fig. 1, A), which starts with the appearance of a flag of wilted, discolored

[1] Now Senior Pathologist, Office of Foreign Agricultural Relations, United States Department of Agriculture. Work performed while employed as Assistant Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Entomologist, Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture.

leaves in the top of the tree, followed almost at once by general wilting, discoloration, and eventual loss of most of the leaves. Rapid defoliation is one of the characteristics of the disease, 2 months often being enough for the infection and death of the entire tree. A less common symptom is the appearance in the spring, on an occasional tree, of small, chlorotic leaves. Such trees sometimes wilt late in the season, but usually the decline is

FIG. 1. A. Wilting persimmon tree on left, healthy tree on right. B. Brownish-black streaks in the wood. C. Spores of *C. diospyri* produced between the bark and wood.

gradual. A tree so affected often lives 1 or 2 years after this symptom appears.

Internally the disease is characterized by the appearance of fine brownish-black streaks throughout the wood of the outer annual rings (Fig. 1, B). At the time the first external symptoms appear, this streaking is present in the younger wood from the roots to the smallest branches and in older parts of the tree in 3 to 10 or more annual rings.

In the fall, orange-pink (3) masses of spores[3] are produced by *Cephalosporium diospyri* beneath the bark of wilt-killed trees (Fig. 1, C). In the smooth-barked parts of the tree, the spores are produced in deep red blisters that rupture and release masses of spores. In the rough-barked portion of the tree, the spores are produced at the bark or tree cambium and usually are not released until the bark starts to disintegrate late in the season. Such bark may easily be pulled or knocked from the tree, releasing clouds of spores. If moisture conditions are suitable, spores will be produced at various points on the tree; even where such conditions do not obtain, spore production almost always occurs at some point near the base of the tree. Occasionally spores will be produced on the dead portion of a dying tree or on a wilted tree that has been felled recently.

HOSTS

Of the two species of *Diospyros* commonly occurring within the limits of the continental United States, the wilt disease is known to attack only the American persimmon, *Diospyros virginiana*. A dwarf variety, *D. virginiana* var. *mosieri* (Small) Sarg. (4) (*D. mosieri* Small (5)), is reported as occurring in Florida in the Lake Okeechobee region. When last surveyed in 1938, the wilt disease was not found in this region. Inoculations on seedlings of *D. texana* Scheele, from Texas, indicate that this species is also susceptible to the disease.

A few seedlings of *Diospyros ebenaster* Retz., native to the East Indies but long grown in Mexico and Central America and now considered naturalized in those regions, have been inoculated and found to be fully as susceptible to the wilt disease as is the American persimmon. Symptoms in this species are the same as those in the American persimmon. *D. discolor* Willd., native to the Philippine Islands, *D. rosei* Standley of Mexico, and *D. montana* Roxb. of India proved, when inoculated, to be immune from the disease.

A series of tests (Table 1) on *Diospyros lotus* L. and *D. kaki* L., the two species commonly introduced into the United States from Japan and China, indicated that both are highly resistant to the disease but that *Cephalosporium diospyri* can grow in the tissues of both. Under conditions in the South, where the tests were made, *C. diospyri* soon died out in *D. kaki* but survived at least one season in *D. lotus*, making it or perhaps both species

[3] No perfect stage of *Cephalosporium diospyri* has been found; the use of the term "spore" wherever it occurs in the text refers to the conidial form.

TABLE 1.—*Exotic species of Diospyros inoculated with Cephalosporium diospyri in comparison with D. virginiana*

Species[a]	Trees inoculated	Trees wilted	Reisolated from wilted trees	Unwilted—cut				Trees dead from other causes
				Trees streaked	Reisolated from streaked trees	Trees unstreaked	Reisolated from unstreaked trees	
	Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
D. virginiana[b]	295	46.1[c]	55.0	0.0	.....	30.5	0.0	23.4
D. virginiana var. Kawakami	60	30.0	61.1	0.0	.....	36.7	0.0	33.3
D. lotus	125	4.0	40.0	38.4	29.1	36.8	0.0	20.8
D. kaki	47	4.2	0.0	8.5	0.0	72.5	0.0	14.8
D. kaki grafted on *D. virginiana*	73	15.1	81.9	53.5[d]	51.3	16.4	8.3	15.0
D. kaki grafted on *D. lotus*	32	0.0	.....	25.0	25.0	53.1	5.8	21.9
D. ebenaster	2	100.0	100.0	0.0	.....	0.0	.....	0.0
D. texana	17	29.0	0.0[e]	0.0	.....	0.0	.....	71.0

[a] Inoculations failed entirely on *D. rosei* Standley of Mexico, *D. montana* Roxb. of India, and *D. discolor* Willd. from the Philippines.

[b] In the group of *D. virginiana* were included nursery-grown stock from seed produced in Texas, Tennessee, Florida, and Indiana; there being no difference in susceptibility, all were grouped together.

[c] The native persimmon, although almost 100 per cent susceptible in nature, showed a susceptibility of less than 50 per cent when recently transplanted stock was used. This indicates that it would be relatively easy to misinterpret the results of experiments on susceptibility to a vascular disease where the tests are conducted on recently transplanted stock.

[d] Reisolation in these cases was 15.4 per cent from unstreaked portions of the same tree.

[e] The small size of the stems of the trees used caused them to dry out shortly after wilting and made reisolation impossible.

possible suspects as carriers of the disease. One case was found in Florida and one in South Carolina in which orchard trees of *D. kaki* growing on native persimmon root stocks died of wilt after the root stock became infected, either directly or after the fungus grew through the *D. kaki* portion of the tree into the root. Both cases have been demonstrated experimentally. Minute quantities of spores of *C. diospyri* were found in the *D. kaki* top of the trees in Florida.

DISTRIBUTION

A factor in the spread of the wilt fungus seems to be the density and distribution of the persimmon population. Because to a great extent spread from tree to tree is a matter of chance, availability of the host is apparently one of the important factors in determining the pattern of its geographical advance (Fig. 2).

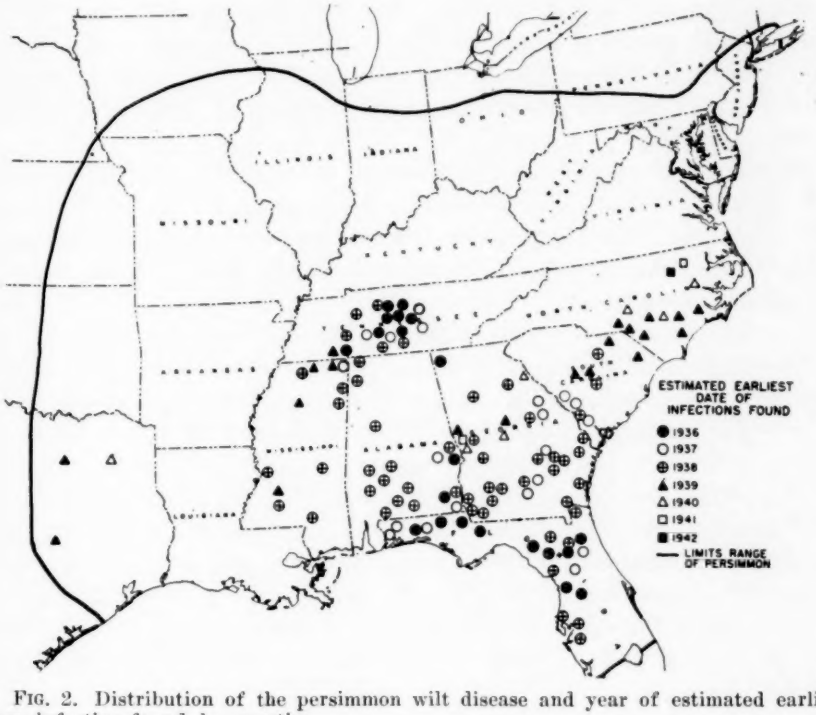

FIG. 2. Distribution of the persimmon wilt disease and year of estimated earliest known infection found, by counties.

During the period under observation the disease has continued to be of spotty distribution, clumps of trees remaining healthy for a number of years even though adjacent to infected trees, while new infections appear miles away from the nearest tree known to harbor the disease.

Because of the rapid deterioration of wilt-killed trees, caused by the fungus *Schizophyllum commune* Fr., it has been impossible to definitely place any infections at a date earlier than 1936. Trees that die of wilt in

the summer begin to disintegrate within 2 years. The first complete survey was made in 1938, 1936 being the earliest year in which dying could be accurately placed. This survey indicated three centers of infection. Either of the apparent centers in Tennessee or Florida could be suspected of being the original point of entry of the fungus, if it is of introduced origin. Each has an early history of attempts to introduce exotic persimmon species. Reports of residents near infections in Tennessee date the appearance of the disease as early as 1934 and, in one doubtful case, 1931. There has been little apparent extension from this center, which contained seven areas on which trees died as early as 1936, except to the southeast and southwest. Within the area, however, the disease when last observed in 1948 had continued unabated.

A nurseryman in the west Florida area stated that persimmons had been dying there since 1928 or 1929. There were found in this region seven areas in which trees died as early as 1936. It is from this group that the fungus seems to have extended across Georgia and South Carolina and well into North Carolina. Observations in 1948 indicate that the disease, for reasons not understood, has not persisted in North Carolina or north of the middle of South Carolina.

Forest Service personnel had observed persimmon trees dying before 1936, in north-central Florida. In this region seven areas, which dated to 1936, were found. From these the disease apparently has extended southward, but movement to the north apparently was limited by an area containing few persimmons.

The wilt disease was reported from Texas in 1939 (2).

On the basis of the evidence available, it appears impossible to determine the point of entry of the fungus, assuming that it was introduced, as its behavior suggests. No clues have thus far been found as to its origin.

No published reference found mentions a similar disease or a *Cephalosporium* attacking any member of the ebony family. The fungus apparently got its start before 1930. The fact that all isolations thus far made have yielded strains of *C. diospyri* indistinguishable from one another indicates that perhaps only one introduction occurred, or at least that only one found a susceptible host. The ability of dry spores to remain viable for as long as 8 months makes it quite possible for the fungus to have been introduced.

ETIOLOGY

Isolations

Sections from the branches, trunk, and roots of dying persimmon trees were collected and chips from the streaked portions of the wood of each were embedded in plates of malt agar. In almost every case a pinkish fungus grew from the bits of wood. This fungus was found to be a species of *Cephalosporium*, later described and named *C. diospyri* (1). Numerous cultures, made in the early fall from spores produced on wilt-killed trees,

TABLE 2.—*Relationship of season of inoculation to development of symptoms of persimmon wilt*

Developmental stage and number of trees inoculated[a]	Date of inoculation (1938)	Progress of wilt in trees after inoculation									
		Aug 1938	Oct. 1938	Winter 1938–39	Feb. 1939	Spring 1939		Aug. 1939	Oct. 1939	Winter 1939–40	Spring 1940
		Trees wilting	Trees dead, spores	Trees dead, no spores	Trees infected	Trees dead, no leaf symptoms	Trees dead, small-leaf symptoms	Trees wilted	Trees dead, spores	Trees dead, no spores	Trees apparently healthy
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Dormant period											
30	Feb. 24	94	87	13							0
56	Mar. 22	97	91	7	0						2
Active growth period											
59	May 11	95	90	7	0						3
25	May 26	100	64	16	20	20					0
15	June 15	93	40	27	27	27					6
20	July 12	40	0	0	100	5	95	0	40	55	0
47	Aug. 15	0	0	0	98	0	62	36	72	17	9[b]
8	Aug. 23	0	0	0	100	0	100	0	100		0
30	July 28	0	0	2	74	0	47	43	57	33	10
Hardening-off period											
13	Oct. 11	–	0	0	0	0	0	23	8	23	69
Dormant period											
20	Nov. 15	–	–	0	0	0	0	90	55	25	20[c]

[a] Eighty-five check trees were included with the inoculated trees. These were treated just as were the inoculated trees, with the exception that no inoculum was administered. Wounds on these and the inoculated trees were left uncovered. Eight of the 85 checks became infected during the course of the experiment as contrasted with 304 of the 323 inoculated trees that became infected.

[b] Some of these died during the summer of 1940.

[c] Three of the four trees were infected and died during the summer of 1940.

were identical with those resulting from tissue isolations. During the course of the surveys for this disease either tissue or spore cultures were made from material in each new location. Comparison of these cultures with cultures from Texas and isolates from the naturally infected *Diospyros kaki* found in Florida failed to demonstrate any measurable variation among isolates of *C. diospyri.*

Inoculations

Shortly after isolating the *Cephalosporium* suspected of causing the disease, a small inoculation experiment was started in June 1937, which proved the pathogenicity of this fungus. However, while this demonstrated the

TABLE 3.—*Comparison of different methods of artificially inoculating persimmon trees with atomized spore suspensions of Cephalosporium diospyri*

Inoculation	Type or place of inoculation	Trees inoculated	Trees infected
		Number	*Per cent*
To simulate injury by:			
Tunnels of *Xylobiops* sp. and *Neoclytus* sp.	$\frac{1}{16}$-in. hole to cambium, tree dormant	17	94
	$\frac{1}{16}$-in. hole to xylem, tree dormant	57	81
	$\frac{1}{16}$-in. hole to xylem, tree active	86	99
Stubs left by twig girdler *Oncideres* sp.	Branch ends cut off, tree dormant	45	91
	Branch ends cut off, tree active	45	91
Feeding scars of *Oncideres* sp.	¼-in. circle of bark scraped off to xylem, tree active	42	100
	¼-in. circle of bark scraped to inner bark on cambium, tree active	55	53
	¼-in. circle of bark scraped off to xylem, tree active, inoculated 24 hr. later	20	65
Cattle	Leaves and small twigs wounded by rubbing, tree active	20	15
Caterpillar	Leaves stripped from branch, tree active	20	95
Insect holes	Punctured holes in fruits, tree active	24	0
Pollination	Flower clusters, uninjured, tree active	8	0
Checks	Leaves and twigs, tree active	20	0
	Junction of leaf and twig, tree active	60	0
	Limb shedding leaves in fall, tree dormant	17	0
	Branch shedding twigs in fall, tree dormant	10	0

pathogenicity of *C. diospyri* isolated from the streaked wood of wilting persimmon trees, it did not duplicate all of the symptoms found in nature. A second inoculation experiment was therefore run, again demonstrating the pathogenicity of *C. diospyri*, but this time producing all the symptoms that occurred in nature. Table 2 shows that the different symptoms of the disease as they appear in nature are the result of the trees becoming infected at different times in the growing season. In the majority of naturally occurring cases the trees suddenly wilted and died, producing spores by late

summer or fall, but in a few cases the trees came out in the early spring with small chlorotic leaves. By varying the time of inoculation, each of the symptoms was produced. The trees used for the inoculations summarized in table 2 varied from ¾ to 1½ in. in diameter. This was a size of tree commonly found affected with the disease in middle Tennessee. Trees were inoculated by atomizing a *C. diospyri* spore suspension in water into two 1/16-in. holes drilled through the bark into the wood or on the stub remaining when a branch end was cut off. The two methods worked equally well (Table 3). Subcultures of one of the original isolations of *C. diospyri* made in June 1937 have been used in all inoculation studies. No decrease in virulence was noted over the 4-year period in which it was kept in culture.

In addition to the inoculations on the native persimmon summarized in table 2, nursery-grown trees of the native persimmon were compared with representatives of such exotic species of persimmon as were available in a series of inoculation tests. Included in this test were seedlings of Kawakami, a supposed hybrid between the native persimmon and *Diospyros kaki* but listed in table 1 as a variety of *D. virginiana*, and selections of *D. kaki* growing on root stocks of *D. lotus* and *D. virginiana*. The results of these inoculations are summarized in table 1. Most significant of the findings were that three of the four Asiatic species tested were resistant or immune from the disease, and that in *D. kaki* it was possible for the fungus to be present without any symptoms being manifest. The variety Kawakami, if a hybrid, which is doubtful, is as susceptible to the disease as is the native persimmon.

Temperature Requirements and Viability of Cephalosporium diospyri

In the course of the investigational work on persimmon wilt, sections of spore-bearing bark and wood were collected, placed in dry Petri plates, and stored at room temperature. From time to time cultures were made from spores from these sections. At the end of 8 months viable spores were still present in the samples, but the experiment was abandoned at that time because of the increasing abundance of molds in the cultures. Pure cultures of the organism have been kept for 6 months through the summers in Tennessee and Georgia, and for at least 4 months the cultures were completely dry. No difficulty was encountered in finding viable spores after this length of time.

In a controlled test to determine the temperature requirements of *Cephalosporium diospyri*, one isolate from Tennessee and one from Florida were used. Spores of the two isolates were inoculated onto malt agar plates and the plates were placed immediately in controlled temperature chambers at 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40° Centigrade. *C. diospyri* had a very narrow growing range: growth was slight at 20° C. (68° F.), good at 25° C. (77° F.), and best at 30° C. (86° F.). Above or below these temperatures the fungus did not grow. The spores that had not germinated

when held below 20° C. started growing as soon as the plates were transferred to a favorable temperature, but above 30° C. no growth occurred after removal from the chambers. Under the moist conditions in the plates, the spores had started to germinate and the mycelium was then killed. It is known that dry spores can live in temperatures as high as any in the series. In the vicinity of Washington, D. C., cultures of the fungus on sticks of autoclaved persimmon in large test tubes were placed in the open in the fall, as soon as plentiful spore production had occurred. During the winter the cultures were exposed to temperatures well below freezing. In the spring viable spores were found in the cultures.

EPIDEMIOLOGY

Transmission

During the first 2 years of investigation on the wilt disease several hundred trees in all stages of wilting were dissected in an effort to determine how the causal fungus gained entrance. These efforts were fruitless in all cases, since *Cephalosporium diospyri* was always found throughout the trees, never being confined to trunks, limbs, or roots. Wood removed from any portion of a diseased tree was uniformly streaked, and cultures taken from any part of the streaked wood yielded the pathogen. It was demonstrated early in the work, when spore suspensions were sprayed into wounds of various types and onto unbroken leaf and twig surfaces (Table 3), that a wound was necessary for primary infection.

When it was established that the fungus could enter healthy trees through wounds, persimmon was surveyed and the agencies responsible for wounds were classified. Trees of all ages were examined, some in great detail. Included were trees growing as isolated individuals and in dense stands. Altogether several thousand trees were so examined. With minor exceptions it was possible to classify wounds, according to the agencies responsible, into three groups: 1) wounds in reproduction, caused by browsing cattle; 2) wounds caused by winds and storms in trees of all sizes but principally in large ones; and 3) wounds caused by insects in trees of all sizes. Injuries by cattle were sometimes abundant in pasture-grown reproduction.

Upon finding that insects had been responsible for a considerable proportion of visible wounds, studies were undertaken to determine the species that had been attacking persimmon. From April through October insects found in and on healthy and diseased trees were collected and identified.

Of the 24 separate species identified, many represented groups inhabiting only dying and dead trees. Interest was greatest in those which attacked healthy trees. Records were kept of the types of insect wounds encountered. These fell into four groups, as follows: 1) small, round holes about $\frac{1}{8}$ in. in diameter, extending through the bark and into the wood; 2) girdled twigs, limbs, and main stems of small diameter, cut so deeply that they

toppled under slight pressure; 3) feeding scars on twigs and small limbs and around terminal buds, made by a chewing insect; and 4) injuries to foliage by defoliating insects. Investigation revealed the small holes through the bark to be the work of the powder-post beetle, *Xylobiops basilaris* (Say), a species normally inhabiting dying and dead trees but which attacks healthy trees when populations are heavy and favored host material is scarce. The girdled twigs, limbs, and main stems, as well as the feeding scars on twigs and limbs and around terminals, all resulted from the work of the twig girdler *Oncideres cingulatus* (Say). Neither of these species confines its activities to persimmon, each having a fairly wide range of host plants in the region of study. Injuries to foliage resulted largely from the work of the fall webworm *Hyphantria cunea* (Drury), which was fairly common on persimmon and a wide variety of other tree species in late September. Occasional colonies of the red-humped caterpillar *Schizura concinna* (A. and S.) were also encountered on persimmon, but they were of little importance.

Female adults of the powder-post beetle excavate tunnels in the wood of dying trees and deposit their eggs in tiny slits along the tunnel walls. Upon hatching, the young larvae tunnel outward from the egg gallery, feeding on the woody tissues as they go. When mature they construct cells at the ends of their tunnels in which they undergo a resting period while transforming to the adult stage. The young adults thereupon excavate a tunnel outward and escape through a shot hole in the surface. Heavily infested persimmon was literally peppered with these emergence holes. Soon after emergence, adults scatter in search of new breeding materials, and as long as a plentiful supply of weakened or dying trees is available they are seldom found on other hosts. Maximum beetle emergence in 1938 occurred in early spring and in the fall, with scattering emergence throughout the summer months.

Although it is true that *Xylobiops* decidedly prefers to attack weakened and dying trees, it can and will attack healthy trees in the absence of weak and dying ones. Seldom, however, are adults able to complete an egg gallery in healthy persimmon before the injured woody tissue begins to throw out a mass of gum, filling the tunnel and forcing the adults either to vacate it or be drowned. Even in rare instances when females remain long enough to deposit their eggs and the eggs succeed in hatching, gum is practically certain to flow into the minute larval tunnels and destroy the larvae. In the majority of attacks observed on healthy trees, adults hardly more than scored the wood beneath the bark before a copious flow of gum forced them out. These attack points were signalized by the presence of jet-black gum droplets, which were easy to see and were observed in large numbers on healthy trees growing in dense clumps containing one or more dead brood trees. In mid-April, 1938, they were very common, indicating a heavy attack late in 1937 or early in 1938. Contrasted with the prevalence of

Xylobiops attacks on near-by healthy trees was the great scarcity of such attacks on healthy trees growing at a distance from brood trees. That adults could fly considerable distances was demonstrated, however, by the speed with which diseased and dying trees growing at considerable distances from brood trees were attacked.

In connection with the studies on *Xylobiops*, efforts were made to isolate *Cephalosporium diospyri* from wood just behind several attack points. Positive cultures were obtained in less than 1 per cent of the samples. Since adults emerging in the fall, and probably in early spring, from wilt-killed trees might encounter fruiting bodies of the wilt fungus, an effort was made to culture *C. diospyri* from the bodies of several dozen collected as they

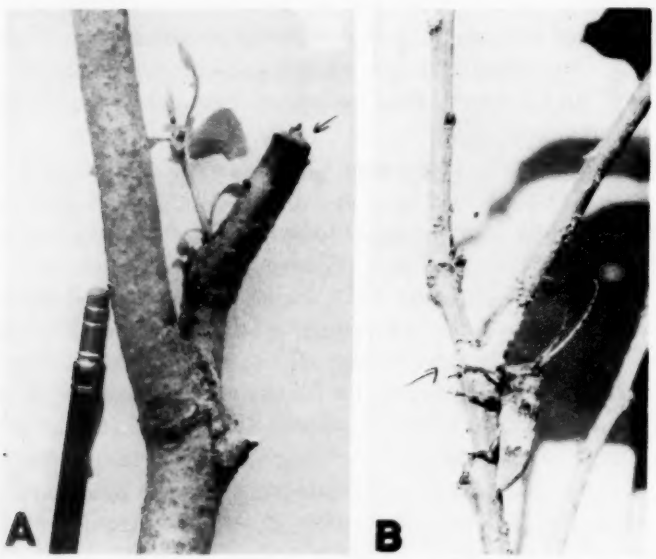

FIG. 3. A. Pruning stub caused by *Oncideres*. B. Feeding of *Oncideres*, showing scar.

emerged from their tunnels. Results were all negative. Further culturing on a more comprehensive scale might yield different results. However, it was obvious that no more than a minute fraction of the wounds made by this species could be contaminated, otherwise the disease would have been far more severe than it actually was in the region.

As indicated previously, one probable reason for the small amount of infection originating at these points is the ability of persimmon to seal over with gum the wounds made in healthy tissue. Unless spores can find entrance into the average wound within a comparatively short time after it is made, they may never be able to do so. However, certain wounds of this nature on any tree gum over very slowly or not at all, depending on obscure factors, one of which probably is the availability of ground moisture. For instance, in one series of wounds similar to those made by *Xylobiops*, made when ground moisture was plentiful, many were sealed over within

24 hr. and all were filled within 5 days. In contrast, when a similar series was made during a drought period late in the season no signs of gum were present in any wound after 17 days. In other words, although the typical response of healthy persimmon when wounded by *Xylobiops* is to exude gum and seal over the wounded surface, this is variable and some wounds may remain exposed to spore entrance for some time.

In the region of investigations in middle Tennessee, the twig girdler, *Oncideres cingulatus*, attacked healthy persimmon trees of all sizes on a widespread scale. Signs of its activities were noticeable throughout the region. The most obvious result of its work was pruned stubs of twigs and limbs (Fig. 3, A) on trees of all sizes, and the main stems of small reproduction. These resulted from the work of the female beetles which, after laying their eggs in slits in the bark of limbs, moved back toward the trunk and girdled the limb, causing it to break and fall to the ground. A second form of injury, very common wherever persimmon was examined, resulted from the beetles' feeding on the bark and often into the wood of the more succulent portions of branches, sometimes to the pith of stems of small diameter (Fig. 3, B).

In a survey of the prevalence of wounds by *Oncideres*, a total of 136 diseased trees of all sizes and 1000 healthy seedlings and sprouts were examined. In the large, mature trees, random samples of limbs and twigs representing several hundred lineal feet were examined in detail. The small trees were examined throughout. Pruned stubs were present on 70 per cent of the diseased trees, and feeding scars on 84 per cent. In the 1000 healthy seedlings, pruned stubs were present on 16 per cent and feeding scars on 35 per cent. There was a close correlation between the presence of pruned stubs and feeding scars on the same trees in the seedlings and sprouts examined. In a sample of 49 such trees, each of which bore one or more pruned stubs, 75 per cent also had been fed upon and bore from 1 to 21 feeding scars. A sample of 50 similar trees, without pruned limbs, showed only 22 per cent with feeding scars ranging in number from one to nine per tree. In the large, mature trees, the full effects of feeding and pruning by this species could be seen. Counts on two trees, each over 100 years old, revealed an average of over 1500 *Oncideres* wounds per tree. This contrasts strongly with an average of 1.7 such wounds per tree in reproduction. This great difference alone in number of wounds between trees in the two size groups of persimmon may go a long way toward explaining the reason for the first appearance of the disease among large trees and the fact that but very few of them were still alive in the study region in 1938.

As is true of wounds made by *Xylobiops*, those made by *Oncideres* are often sealed over with gum and after this occurs fungus spores probably can not gain entrance into the tree. However, again there is wide variation between wounds in rate and degree of gum formation. A series of wounds similar to those produced by feeding adults was made August 11, 1938, at

the start of the active beetle season. After one week, 78 per cent of the wounds had sealed over while the remaining 22 per cent remained dry through October. During September and October, when beetle activity was at its height, and when spores were being produced and liberated in large quantities, hundreds of newly pruned stubs were observed. The large majority of these remained free of pitch for several hours or days. Eventually practically all such stubs were covered with a cap of jet-black gum.

The presence of the fungus in wood just back of 1937 *Oncideres*-pruned stubs was demonstrated in the spring of 1938, when nine positive cultures of *Cephalosporium diospyri* were obtained from a sample of 62 stubs collected from otherwise disease-free trees in a region with plentiful spores. Early in the fall of 1938, as soon as spores were plentiful, cultures were taken from 120 stubs with a streaking in the vessels. These stubs were collected in four separate areas, and all came from trees growing within 200

TABLE 4.—*Comparison of the effect of different types of injury on 3- to 5-in. persimmon trees when exposed to air-borne spores of Cephalosporium diospyri*[a]

Type of injury	Number of trees treated	Percentage infected
Dry spores dusted into ⅛-in. holes extending ½ in. into xylem[b]	18	94.5
Leaves crushed on one-half of each forked branch tip	6	16.6
Leaves stripped from one-half of each forked branch tip	10	30.0
20 twigs cut from each tree at fork nearest branch tip	23	4.3
Trees untouched, clump selected at random	7	14.3

[a] Approximately ½ teaspoon of dry spores, produced in culture, released on the windward side of the trees in clumps.

[b] This treatment served to determine the viability of spores.

yards of wilt-killed trees. Positive cultures were obtained from two stubs, representing less than 2 per cent of the total. Although this percentage seems small, it obviously is large enough to account for the amount of new infections in the region, especially when added to the probabilities of infection in other wounds by the same species, plus those resulting from the work of other agencies. It was demonstrated experimentally that infections could be caused by air-borne spores. Although the experiments themselves were inconclusive, since they failed to demonstrate that any particular type of wound favored entry, they served to show the ease with which persimmons became infected by air-borne spores (Table 4). After 10 cc. of the spores were released to windward of each clump, from 4.3 to 30 per cent of each of three groups of trees wounded mechanically in different ways to simulate injuries occurring naturally, and 14.3 per cent of the control group of supposedly unwounded trees became infected. Natural infection was not present in the immediate vicinity.

From the foregoing discussion of transmission of the disease from tree to tree, it will be seen that many contributing agencies are responsible. Anything that produces a wound in a healthy tree, during the rather long

period when spores of the fungus are released from diseased trees and are being transported by wind or other means, must be looked upon with suspicion. However, from the evidence available after a season's study, it appears that the principal wounding agencies were the two insects *Xylobiops basilaris* and *Oncideres cingulatus*. Yet in pasture-grown reproduction these were often secondary to cattle, which browsed on and bruised and battered the small trees. The full effect of the wind in causing breakages in the bark and in injuring the foliage in such a manner that wind-borne spores could gain entrance was not determined. Disease spread as it normally occurs in the field is a result of the effect of all wounding agencies, the two insect species mentioned above being principally suspected.

Pathological Anatomy

Occasionally in nature a tree is found that, instead of wilting, produces small chlorotic leaves and lingers for 2 or 3 years before dying. It has been demonstrated experimentally (Table 2) that this symptom is shown by trees that became infected at the height of the growing season. Such trees apparently become infected too late in the season for wilt symptoms to manifest themselves, and the small-leaf symptom then appears in the next growing season. Experimentally inoculated trees (Table 2) died by the end of the third growing season following inoculation. In a replication of this experiment, with 12 larger trees, all showed slight wilt the first season, one died during the winter, and the remaining 11 showed typical small-leaf symptoms the following growing season. Of these, three died the following winter. The surviving 8 again came out with small leaves but survived, although they made almost no growth. The wilt fungus remained alive in at least part of these. Three more died during the next winter. The remaining trees again came out with small leaves, but in apparently no worse condition than in the season before. In these five cases the fungus could still be recovered from the streaked wood.

Based on cultural evidence in inoculated trees, if the fungus enters the tree at the beginning of the growing season it is present throughout the tree within a month. When entry is made at the end of the growing season, the fungus apparently does not start its spread through the tree until the tree itself starts to make its new growth. Little evidence is available on whether the fungus overwinters as an ungerminated spore at the original point of entry or whether it germinates and makes a small amount of growth and then lies dormant until the resumption of growth in the spring. As previously mentioned, a number of incipient infections that had not yet reached the main body of the tree were found behind pruning stubs of *Oncideres* sp. Obviously any ungerminated spores resting in such openings would not have been detected. Trees artificially inoculated in November did not show any internal symptoms of infection in February but were streaked throughout in May and the trees wilted in June and July.

Once in the tree at a time when growth can take place, the fungus rapidly invades the large vessels of the xylem. In all probability, since the spread is so rapid through the vessels, it takes place by means of the transporting by the vessels themselves of spores produced inside the tree. It has not, however, been possible to prove this in the case of the persimmon wilt fungus, although such a process is known to occur in other closely related vascular parasites. It is doubtful that much spread occurs from the original inoculation, since the very nature of the entry into broken vessels would preclude much transpiration taking place in them. Within a few weeks of the time of entry, the fungus is present in small trees in all but the inner one or two rings, and often it is found in the outer three or four rings of a 5-year-old tree. In older trees that have been dissected, there seems to be no definite limit where it is found. On one tree over 100 years old, it was found in the outer 25 annual rings, and while this is exceptional, it is common to find it in the ten outer annual rings of 8- and 10-in. diameter trees. In the invaded vessels, the fungus itself causes a light brown discoloration. In trees in early stages of infection, this results in a brown streaking in the wood, not limited to any particular annual ring. Wilting apparently occurs when the tree, as a result of irritation by the fungus or in response to an irritant produced by it, produces gum in the water-conducting vessels. At the time wilting occurs, these vessels are completely plugged with the gum. The wood of such a tree is typically heavily streaked with the black streaks of the gum predominating over the brownish ones found earlier. This gum is commonly produced by the persimmon wherever an injury occurs. In external wounds it serves to protect the tree, but in the case of the wilt disease it appears that the tree's means of protection actually is the immediate cause of its death.

With favorable moisture conditions, as soon as the tree (or in some cases a portion of the tree) dies, spore production occurs. Spore heads of *Cephalosporium diospyri* are produced in abundance at the cambial region, both of the tree and the bark. In the smooth-bark portion in the upper part of the tree the presence of spores can be detected by red blisters caused by the masses of spores forcing the bark away from the wood. In the rough-bark basal part of the tree, the entire bark is loosened, sometimes at the tree cambium and at other times at the bark cambium. In either case the loosening bark reveals large quantities of spores. These spores are produced from August until well into the fall. They are almost always present, at least near the base of the tree. Sometimes, if the weather is sufficiently moist, they are produced throughout the tree. Wilt-infected trees, cut and left on the ground, will produce spores if a period of wet weather occurs shortly after they are cut.

Sticks of wood taken from the native persimmon, from the wilt-resistant Chinese and Japanese species, and from hickory have been placed in test tubes, sterilized, and inoculated with the persimmon wilt fungus. Abun-

dant spore production occurred on the sticks from American persimmon, little or none on the sticks from the Chinese and Japanese species, and a fair amount on the hickory sticks. In all cases where spores were produced, the fungus grew up inside the stick and spores were produced under the bark, causing typical blisters.

From the evidence presented by inoculations (Table 2) and from field observations, it appears that the wilt fungus gains entry to the tree at some time near the end of the growing season, during the dormant season, or, less likely considering the disease as it typically appears in nature, at the beginning of the growing season. The most likely time for entry, on the basis of prevalence of wounds, is at the end of the growing season and during the dormant season. It has been demonstrated that this does occur in nature. At this time an abundant crop of spores is released. Opportunity for entry is present until well into the winter, since branches girdled by *Oncideres* often do not break from the tree for months after the girdling takes place. The spores remain viable at least until the beginning of the next growing season. Incidental injury by cattle and man occurs at any time. Although it appears that infections probably take place, with few exceptions, during or immediately before or after the dormant period, most of the infections probably start at the end of the growing season and beginning of the dormant period, at the time when the greatest abundance of spores is available.

CONTROL

Although characteristics of occurrence and rate of spread of the disease indicate the possibility of preventing the development of epidemic disease conditions and of retarding normal disease spread, studies of wound-inducing agencies indicate the difficulty of eradicating it after it is firmly established in a region, or of preventing spread altogether. Locally the prevention of mass emergence by *Xylobiops* adults from dead trees by the removal of infected wood prior to adult emergence might very well prove of value in prolonging the lives of near-by trees. Likewise, the collection and destruction of pruned limbs, before adult emergence in late August and early fall, should aid in controlling the twig girdler *Oncideres cingulatus*. Control of these species should materially reduce the number of wounds to be found in persimmon, and this should be reflected in fewer diseased trees in the region. Their complete control on persimmon would not, however, remove them entirely since both species are able to breed successfully in other tree species that commonly occur in the region.

Removal of all trees showing internal streaking as soon as the first wilting tree is noticed will help to eliminate the disease locally but can not, of course, protect against reinfection from outside sources. If the disease has been present more than one year, and is not found until the second or a subsequent year, it is necessary to remove all dead spore-bearing trees and completely destroy them, with as little disturbance to the spore masses as

possible. All infected trees should also be cut and destroyed. Soon after the beginning of the next growing season the trees should again be examined and all infected trees removed. Where a single tree is infected in the trunk, there is nothing that can be done to preserve it. It is best to remove it while it is still green and easy to cut up. Dry persimmon wood is very tough and hard. It should be remembered that it is a habit of persimmon to grow in clumps, all trees in a particular clump often coming from one root system of an original tree that has been destroyed. All trees in a clump may or may not be interconnected. While all trees in a clump should not be eradicated because one tree becomes infected, neither should the person attempting eradication become discouraged if tree after tree wilts and dies in spite of removal of infected trees. All may be part of the original infection and removal of each as it shows symptoms, and before spores are produced, will eventually result in removal of all trees infected.

Experimentally it has been possible to clear an area of persimmon and to create a neutral zone around healthy trees by cutting the trees to be removed at the ground line, just after active growth had started, and then spraying the stumps with a water suspension of spores. Such stumps sprouted but the sprouts died by late summer without any spores being produced. A year later there was no evidence present that persimmon had ever grown on the cleared area.

SUMMARY

In 1937 a hitherto undescribed wilt disease of the native American persimmon was discovered in Tennessee. The cause of the disease was found to be a fungus, *Cephalosporium diospyri* Crandall. The pathogenicity of this fungus was proved by more than 500 successful inoculations into naturally growing persimmon trees. The disease itself is unusual, in that it is apparently the fastest-killing disease of vigorous and healthy trees on record. A scouting program started soon after discovery of the disease showed it to be established in seven southeastern States. Observations made in 1948 indicate that it still persists in all these States except North Carolina. Isolated cases have been found in Texas. Because of the widespread distribution, general control was not suggested or attempted. In individual limited experimental tests it has been possible to eradicate the disease. Because of the epidemic nature of the disease it was suspected of being introduced, although this has not been proved. Because of the high degree of resistance of the Asiatic persimmons *Diospyros kaki* and *D. lotus,* they are ranking suspects on the list of possible carriers of the disease by which it may have been introduced. *D. discolor,* native to the Philippines, *D. rosei* of Mexico, and *D. montana* of India are apparently immune, whereas *D. ebenaster,* an East-Indian species, is susceptible. *D. texana,* of the southwestern United States and Mexico, is apparently susceptible.

The fungus gains entrance into healthy trees through wounds made by various agencies. Special studies of insects attacking persimmon, in their

relation to disease spread, indicate that two species of beetles, *Xylobiops basilaris* (Say) and *Oncideres cingulatus* (Say), were responsible for the vast majority of wounds in healthy trees produced by this agency. The fungus was artificially established without difficulty in wounds similar to those caused by the two species. It was also cultured from wood taken from immediately behind wounds made by the species. Higher disease incidence was found in wood collected from behind wounds made by *Oncideres*. Field observations of the species *X. basilaris* and *O. cingulatus* showed that both were most abundant and actively wounding trees when wilt-killed trees were liberating plentiful quantities of spores.

In nature the disease is typically a fast-killing wilt, characterized by brownish-black streaks throughout the outer annual rings of wilted trees. As soon as death occurs, spores of *C. diospyri* are produced in large quantities between the bark and wood near the base of the tree. These masses of spores cause the bark to separate from the wood and eventually fall from the tree. In the smooth-bark portion of the tree, spores are often produced under the bark, causing red blisters, and are released when pressure causes the bark to rupture.

U. S. DEPARTMENT OF AGRICULTURE

LITERATURE CITED

1. CRANDALL, BOWEN S. A new species of Cephalosporium causing persimmon wilt. Mycologia 37: 495–498. 1945.
2. DUNLAP, A. A. Cephalosporium wilt of persimmon in Texas. U. S. Dept. Agr., Pl. Dis. Reptr. 23: 347. 1939.
3. RIDGWAY, ROBERT. Color standards and color nomenclature. 43 pp. and 53 color plates. Washington, D. C. 1912.
4. SARGENT, CHARLES S. Manual of the trees of North America. 2nd edition. 823 pp. 1933.
5. SMALL, J. K. Old trails and new discoveries. N. Y. Bot. Garden Jour. 22: 25–40. 1921.

RELATION OF CONCENTRATION OF SOME ORGANIC SUBSTANCES TO SPORE GERMINATION AND DOSAGE RESPONSE

HAROLD J. MILLER[1]

(Accepted for publication August 28, 1949)

McCallan and Wilcoxon (3) noted the effect of variable amounts of the culture substrate on spore germination in a copper sulphate solution. Dimond *et al.* (2) obtained an increased LD50 when 0.1 per cent orange juice was added to noncentrifuged spore suspensions of *Macrosporium* (*Stemphylium*) *sarcinaeforme* with red cuprous oxide. McCallan and Wellman (4) pointed out that orange juice increased the resistance of spores to certain chemicals. Miller (5) reported 1) that different lots of orange juice gave different LD50 values on a Bordeaux mixture deposit, and 2) that a linear relationship exists between orange juice concentration and LD50 values. This was also true for sodium and potassium citrates. The role of extracts, citrate compounds, and sugars in spore germination has been summarized (6). The data reported here show the effect of varying the concentration of these materials on LD50 and spore germination.

METHODS

The method of using centrifuged suspensions of the conidia of *Sclerotinia fructicola, Alternaria oleracea,* and *Venturia inaequalis,* and the swarmspores of *Phytophthora infestans* on dried fungicide deposits on Pyralin slides, has been described (1, 6). The orange juice was prepared according to the recommended procedure (1) and the Lima-bean extract was obtained by soaking the Difco preparation for several hours and then filtering through coarse filter paper.

DOSAGE RESPONSE

Figure 1 (upper) shows the linear relationship between the concentration of orange juice and LD50 values on a Bordeaux mixture deposit with *Sclerotinia fructicola* and *Venturia inaequalis,* and of Lima-bean extract with *Phytophthora infestans.* The curves for sucrose and dextrose with *P. infestans* and *S. fructicola,* respectively, did not show any change in LD50 with increasing concentrations comparable to that with orange juice and citrate. The relationship of LD50 values on a Bordeaux mixture deposit to the concentration of citrate is shown to be linear with the four different organisms for both the potassium and sodium salts, as well as for

[1] Authorized for publication on Nov. 23, 1949, as paper No. 1560 in the journal series of the Pennsylvania Agricultural Experiment Station and as Contribution No. 157 from the Department of Botany. This work was completed while the author was Assistant Professor of Plant Pathology, Pennsylvania State Agricultural Experiment Station, State College, Pa. The author's present address is Pennsylvania Salt Manufacturing Company, Whitemarsh Research Laboratories, Box 4388, Chestnut Hill P. O., Philadelphia 18, Pa.

the citric acid with *S. fructicola* and *V. inaequalis* in figure 1 (lower). The pH values for the citric acid varied from 5.86 at the lowest concentration to 3.32 at the highest as compared to 6.10 to 6.84 for the citrates.

The relationship of other metal compounds is given in figure 2. Copper oxychloride, copper sulphate, and nickel hydrate gave the linear response to

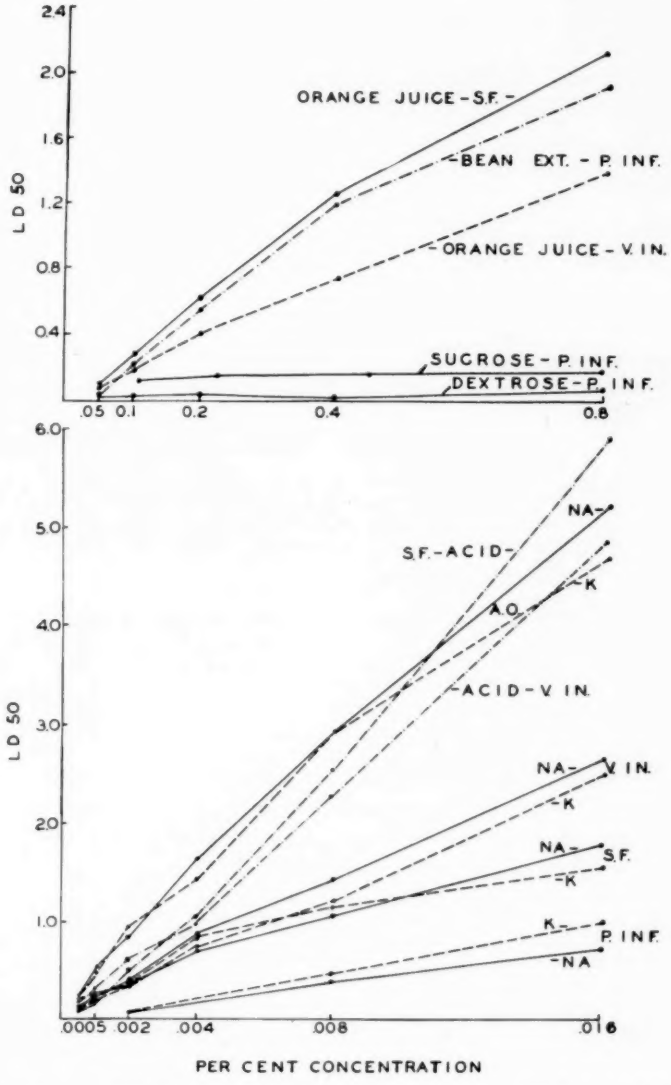

FIG. 1. Upper: Relation of LD50 (actual copper) of a Bordeaux mixture deposit to the concentration of orange juice with *Sclerotinia fructicola* (S. f.) and *Venturia inaequalis* (V. in.), extract of Lima-bean agar with *Phytophthora infestans* (P. inf.), sucrose (plus 0.005 per cent potassium citrate) and dextrose with *P. infestans*. Lower: Relation of LD50 of a Bordeaux mixture deposit and concentration of sodium and potassium citrate with *S. fructicola*, *V. inaequalis*, *Alternaria oleracea* (A. o.), and *P. infestans* and citric acid with *S. fructicola* and *V. inaequalis*.

varying concentrations of potassium citrate with *Sclerotinia fructicola*. The zinc sulphate tended to flatten off at the higher concentrations of citrate. Cadmium dinicotine thiocyanate, mercuric chloride, and potassium dichromate showed no increased LD50 values with higher concentrations of the citrate.

No positive relationship could be established between potassium citrate concentration and LD50 values of these organic compounds (Fig. 3): copper 8 quinolinolate, dichloronaphthoquinone (Phygon), 2-heptadecyl imidazoline (341), ferric dimethyl dithiocarbamate (Fermate), tetrachlorobenzoquinone (Spergon), sodium ethylene bisdithiocarbamate (Dithane D14), and sodium dinitro ortho cresylate (Elgetol). Apparently the

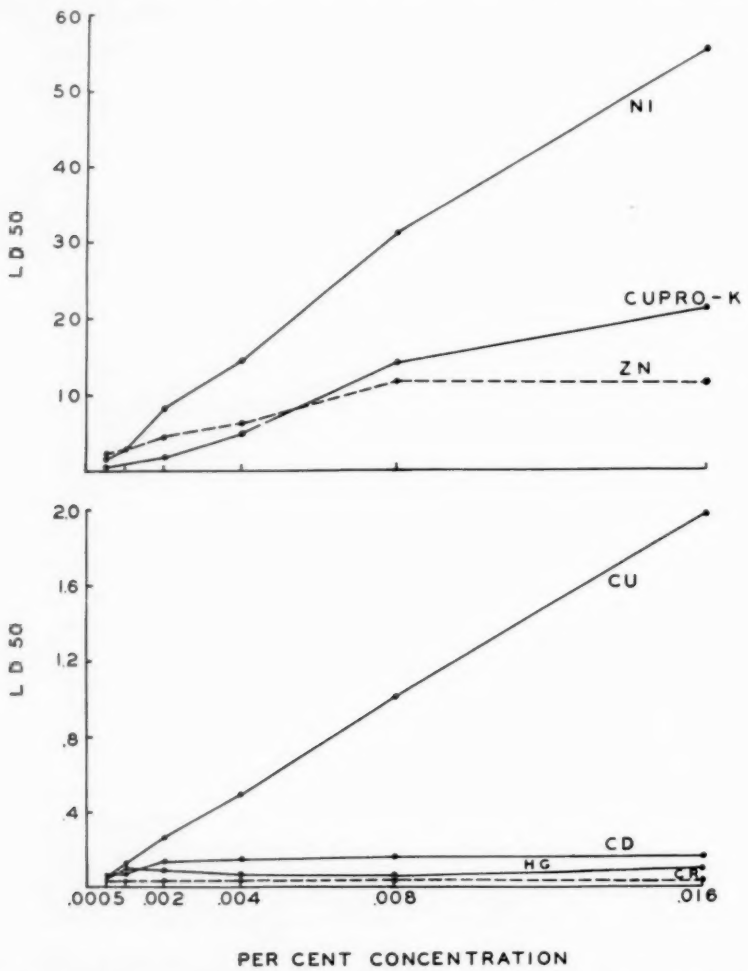

FIG. 2. Relation of LD50 (actual metal) of copper sulfate (CU), copper oxychloride (Cupro-K), nickel hydrate (NI), zinc sulphate (ZN), cadmium dinicotine thiocyanate (CD), mercuric chloride (HG), and potassium dichromate (CR) to the concentration of potassium citrate with *Sclerotinia fructicola*.

copper in copper 8 quinolinolate was masked by the organic part of the compound.

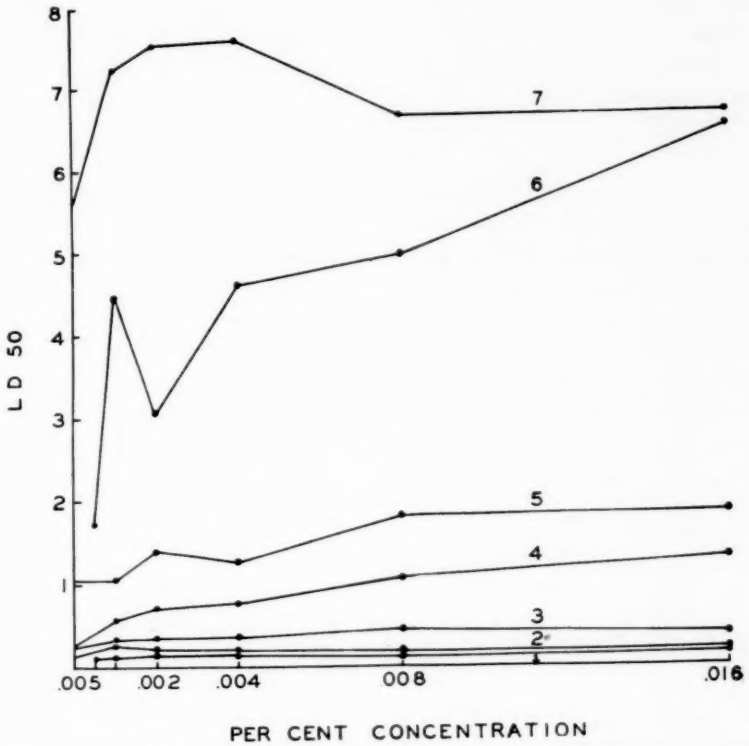

FIG. 3. Relation of LD50 of copper 8 quinolinolate (1), dichloronaphthoquinone (2), 2-heptadecyl imidazoline (3), ferric dimethyl dithiocarbamate (4), tetrachlorobenzoquinone (5), sodium ethylene bisdithiocarbamate (6), and sodium dinitro ortho cresylate (7) to the concentration of potassium citrate with *Sclerotinia fructicola*.

SPORE GERMINATION

The effect of varying concentrations of potassium and sodium citrate and citric and malic acids on the percentage germination is shown in figure 4. Relatively little difference was found with *Sclerotinia fructicola* except a slight reduction at the lowest (0.0005 per cent) and highest (0.032 per cent) concentrations. Malic and citric acids at concentrations above 0.008 per cent reduced the germination of *Venturia inaequalis*. These acids inhibited germination of *Alternaria oleracea* spores. *Phytophthora infestans* swarmspores germinated best with the potassium citrate at 0.004 per cent. Lower germination resulted with sodium citrate, while malic and citric acids at concentrations above 0.005 per cent completely inhibited germ tube formation.

Some increase in germination of *Phytophthora infestans* occurred at higher concentrations of suerose, and of *Sclerotinia fructicola* at higher concentrations of dextrose (Fig. 5). The orange juice increased the ger-

mination of *S. fructicola* but at concentrations above 0.1 per cent germination of *Venturia inaequalis* was lowered. *P. infestans* did not respond to orange juice, and germination was less at concentrations of 0.1 per cent or more. Increasing concentrations of bean extract markedly promoted germination of *P. infestans*.

DISCUSSION

From the data it is evident that the concentration of the materials used in slide germination tests is important in relation to the LD50 values of certain materials as well as in promoting good germination of spores. This certainly should be considered in slide germination tests, and careful adjustments to a standard concentration should be made where such metals as copper, nickel, and zinc are involved.

The work of Sinclair *et al.* (7) is interesting in this connection. They found the citric acid content of oranges to vary from 8.38 to 25.39 mg. per ml. in the same variety. Thus, 0.000838 to 0.002539 per cent of actual citric acid would be contained in the 0.1 per cent orange juice required to give an

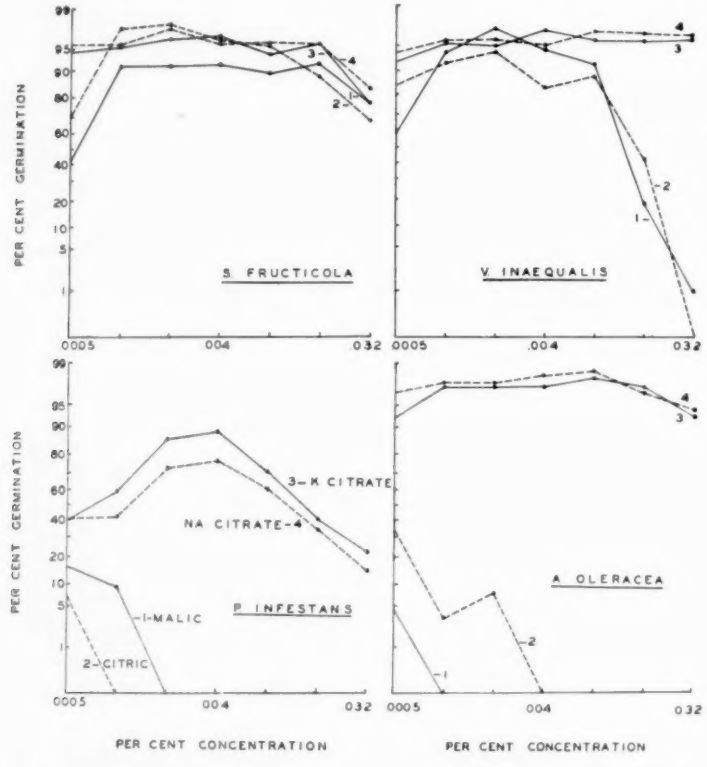

FIG. 4. Relation of concentration of potassium and sodium citrates, and malic and citric acids to spore germination of *Sclerotinia fructicola, Venturia inaequalis, Alternaria oleracea,* and *Phytophthora infestans.* Germination in probits and concentration in logarithms.

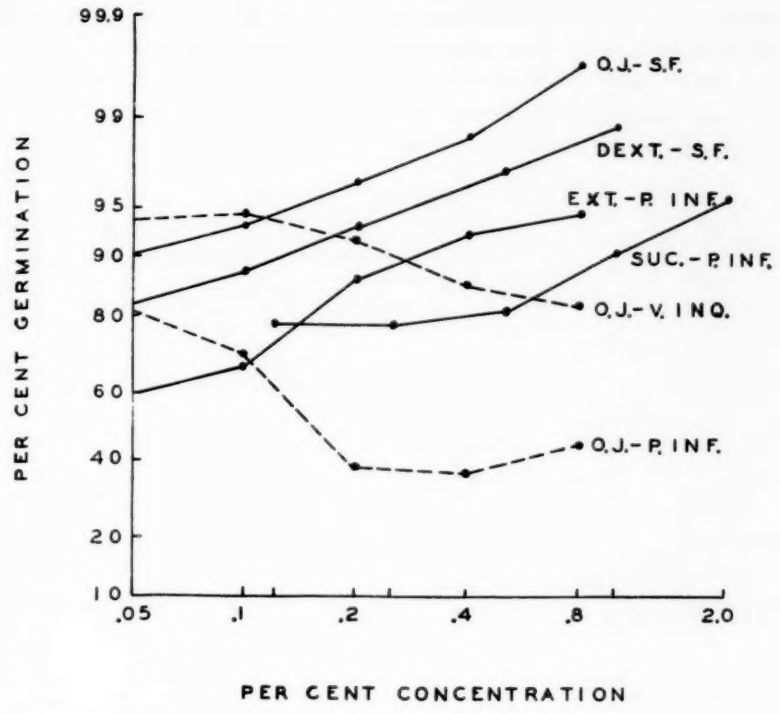

FIG. 5. Relation of concentration to spore germination of *Sclerotinia fructicola* and *Venturia inaequalis* with orange juice. *Sclerotinia fructicola* with dextrose, *Phytophthora infestans* with extract of Difco Lima-bean agar and sucrose (plus 0.005 per cent potassium citrate). Concentration in logarithms and germination in probits.

LD50 value equivalent to that found for 0.001 per cent citric acid. The variable effect of orange juice in different lots could be explained by different citric acid concentrations. It would seem that orange juice contains the necessary citric acid or derivatives as well as sugar required for spore germination and stabilizing the LD50 level.

The nature of this relationship has been suggested by McCallan and Wellman (4) to be an increase in resistance of the spores to certain chemicals when the orange juice is added. A more acceptable theory involves the sequestering action of the citrate molecule on certain metals. This would result in the binding of these ions, thereby requiring more total metal to inhibit spore germination. The absence of any effect on the organic compounds tends to support this view.

SUMMARY

A linear relationship of increasing concentration of orange juice, citric acid and sodium and potassium salts, and bean extract to increased LD50 values of copper, nickel, and zinc compounds was found. Other metallic and organic fungicides produced no such response. The necessity of

standard concentrations of orange juice or citric acid and sucrose for accurate determination of LD50 values is established. The relationship of concentration of citric and malic acids and sodium and potassium citrates to spore germination was also determined.

PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION
STATE COLLEGE, PENNSYLVANIA

LITERATURE CITED

1. COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS. The American Phytopathological Society. The slide-germination method of evaluating fungicides. Phytopath. **33**: 627–632. 1943.
2. DIMOND, A. E., J. G. HORSFALL, J. W. HEUBERGER, and E. M. STODDARD. Role of the dosage-response curve in the evaluation of fungicides. Conn. Agr. Exp. Sta. Bul. 451. 1941.
3. MCCALLAN, S. E. A., and FRANK WILCOXON. An analysis of factors causing variation in spore germination tests of fungicides. I. Methods of obtaining spores. Contrib. Boyce Thompson Inst. **11**: 5–20. 1939.
4. ————, and R. H. WELLMAN. Fungicidal versus fungistatic. Contrib. Boyce Thompson Inst. **12**: 451–464. 1942.
5. MILLER, H. J. The use of Venturia inaequalis and Sclerotinia fructicola with pure chemical stimulants in slide-germination tests of fungicides. (Abstr.) Phytopath. **34**: 1009. 1944.
6. ————. Modifications of the slide-germination method of evaluating fungicides including the use of Venturia inaequalis and Phytophthora infestans. Phytopath. **39**: 245–259. 1949.
7. SINCLAIR, W. B., E. T. BARTHOLOMEW, and R. C. RAMSEY. Analysis of the organic acids of orange juice. Plant Physiol. **20**: 3–18. 1945.

EFFECTIVENESS OF FUNGICIDAL SPRAYS AND DUSTS IN AZALEA PETAL BLIGHT CONTROL

D. L. GILL[1]

(Accepted for publication August 30, 1949)

Azalea petal blight, caused by *Ovulinia azaleae* Weiss, was first observed in the vicinity of Charleston, S. C., by Weiss in 1931 (7). During the next few years it was found along the coastal plain from North Carolina to Texas (8, 9, 10) and in California (4). In 1946 the disease was reported in Takoma Park, Maryland (1), and in 1947 in Norfolk, Virginia (5). Azalea petal blight attacks all varieties of azaleas (various *Rhododendron* spp.) that have been observed. Weiss and Smith (10) have reported several unidentified hybrid rhododendrons as becoming infected when growing interplanted with affected azaleas, and *Rhododendron catawbiense* Michx. and *R. carolinianum* Rehd. as becoming infected following inoculation. They observed the disease on mountain laurel (*Kalmia latifolia* L.) when growing near azaleas. Flowers of *Vaccinium corymbosum* L. and varieties *fuscatum* and *tenellum*, and also those of *Gaylussacia baccata* (Wang.) K. Koch, were experimentally infected. The beauty of affected azalea plants is greatly impaired by the destruction of the flowers. No other part of the plant is affected.

Two eradicant fungicides, calcium cyanamide, 200–800 lb. of the dry granular material per acre, and Elgetol[2], 450 gal. per acre of a 1 per cent solution, were found effective by Brierley in suppressing apothecial development. This unpublished work was abandoned in 1941 because such eradicants would need to be applied on a community basis to eliminate the primary inoculum effectively, as Plakidas (6) has recently pointed out.

Various other control methods were tested by Weiss and Smith (9, 10), but satisfactory control of the disease in gardens was not obtained until 1945 when Westcott (11) reported the successful use of two protectant sprays. These were: (a) Dithane D-14, zinc sulfate (25 per cent zinc), lime, and Triton B-1956; and (b) U. S. Rubber Co. 604, later known as Phygon. Westcott made three spray applications per week. Although growers have obtained good control with the Dithane D-14 spray, some have reported flower injury following its use. Phygon, which Westcott (11) reported as causing slight petal injury, produced severe injury in the writer's tests and in gardens in 1947.

[1] Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Spring Hill, Alabama.

The cooperation of the Alabama State Department of Agriculture, in whose laboratory at Spring Hill this work was conducted, is acknowledged with appreciation.

[2] Composition and sources of fungicides and wetting agents mentioned in this paper may be found in U. S. Department of Agriculture, Plant Disease Reporter, Supplement 181, 1949, except Guantal (diphenyl quanidine phthalate), obtained from Monsanto Chemical Co.; Preventol GD (2,2-dilydroxy 5, 5 dichlorodiphenyl methane), obtained from General Dyestuffs Corp.; and Bloomocide, obtained from Flower Foods, Inc.

The objects of the investigations reported in this paper were to compare additional fungicides and wetting agents, and to determine the strength of fungicide and frequency of application necessary for control. An interpretation of injury resulting from the use of the Dithane D-14 spray is presented. Disease control recommendations for gardeners have been published previously (2, 3).

METHODS

Spraying and dusting tests have been conducted by the writer in the vicinity of Mobile, Alabama, during the past 4 years, along the parkways of the city streets, in nurseries, and in private gardens. Applications of fungicidal materials were begun at the first appearance of the disease, except in 1949, and continued until most of the flowers had fallen. Counts of the diseased flowers were made when untreated flowers were heavily affected. Three applications a week were made unless otherwise stated. In spraying, an attempt was made to hold run-off to a minimum. Sprays were applied in 1946 and 1947 with a Paragon hand sprayer having a 3/64-in. disk in the nozzle. In 1948 and 1949 a Myers Silver Prince motor-driven sprayer delivering 225 lb. pressure was used, with a 1/32-in. disk in the nozzle. Dusts were applied with a Root hand duster and with an American Beauty hand duster. Randomized block design was used in all tests, 2 to 4 plots receiving each treatment. Data were recorded as number of flowers diseased in 400 to 600 counted per plot.

RESULTS

Comparison of Various Fungicides

The Dithane D-14 spray reported by Westcott has continued to give satisfactory control (Tables 1, 2, 3, and 4). This spray consisted of Dithane D-14, 1⅓ qt.; zinc sulfate (25 per cent metallic zinc), 1 lb.; lime, ½ lb.; Triton B-1956, 1 fluid oz.; and water, 100 gal. Unless otherwise stated, the Dithane D-14 sprays used in these tests were made according to the above formula and the zinc sulfate contained 25 per cent metallic zinc. Phygon, 1 lb. in 100 gal. water, as previously recommended (11), has also controlled the disease (Tables 1 and 4).

A number of other materials have been tested for the control of the disease during the past 4 years. Table 1 shows the results of tests with some of these fungicides. In 1946, each material was used upon two plots of Pride of Mobile plants in a lath-house bed. Six hundred flowers were counted per plot. The test was made late in the season when infection was severe. Methasan, Guantal, Spergon, and Glyoxalidine 341 were discarded because they did not offer satisfactory control in comparison with the Dithane D-14 or the Phygon spray. In other 1946 tests, Bioquin 1 and Preventol GD did not provide effective control.

The results of one of two 1947 tests with other materials are given in table 1. Each plot consisted of 10 to 15 Pride of Mobile azaleas growing in

a garden, and each treatment was applied to two plots. Other varieties were present in the same plots, but the data were obtained by counting 400 Pride of Mobile flowers per plot. Puraturf 177, Fermate, Zerlate, and Fermate-Zerlate mixture either did not give effective control or proved objectionable because of residue on the flowers. Tersan gave favorable results in 1947 but failed to give effective control in 1948. Dithane Z-78, 1.44 lb. to 100 gal. water, gave satisfactory control in this and another 1947 test. In a 1947 test (data not shown) Parzate, 1.31 lb. to 100 gal. water, compared favorably with the Dithane D-14 spray.

The 1948 and 1949 tests (Table 1) were made upon clumps of azaleas

TABLE 1.—*Comparison of several fungicides in the control of azalea petal blight, 1946–1949*

Treatment: material and quantity used in 100 gal. water	Mean number diseased flowers per plot			
	1946[a]	1947[b]	1948[c]	1949[c]
Dithane D-14, 1⅓ qt.; zinc sulfate, 1 lb.; lime, ½ lb.; Triton B-1956, 1 fl. oz.	218.0	8.5	5.0	108.8
Phygon, 1 lb.	201.5	13.5		
Spergon, 1 lb.	526.0			
Guantal, 1 lb.; Santomerse 3, 25 gm.	591.5			
Methasan, 1 lb.; Santomerse 3, 25 gm.	422.5			
Glyoxalidine 341, 1¼ lb.	595.0			
Dithane Z-78, 1.44 lb.		36.0	48.5	184.8
Puraturf 177, 1 lb.		84.5		
Tersan, 1⅓ lb.		21.0	101.5	
Fermate, 1⅓ lb.; DuPont Sticker-Spreader, 3 fl. oz.		71.0		
Zerlate, 1⅓ lb.; DuPont Sticker-Spreader, 3 fl. oz.		52.0		
Fermate, ⅔ lb.; Zerlate, ⅔ lb.; DuPont Sticker-Spreader, 3 fl. oz.		35.0		
Phygon XL, 1 lb.			67.0	
Phygon XL, ¾ lb.			118.3	
Phygon XL, ½ lb.			120.0	
Parzate Liquid, 1⅓ qt.; zinc sulfate, 1 lb.; lime, ½ lb.; Triton B-1956, 1 fl. oz.				183.8
Carbide and Carbon No. 658, 2 lb.				324.8
Bloomocide, 1½ pt.				344.8
Untreated	600.0	361.5	363.0	365.5
Least difference between means for significance				
19-1	26.9	34.2	72.6	121.2
99-1	40.8	49.7	96.0	162.1

[a] 600 flowers counted in each of 2 plots per treatment.
[b] 400 flowers counted in each of 2 plots per treatment.
[c] 400 flowers counted in each of 4 plots per treatment.

approximately 10 ft. in diameter and 30 ft. apart along the parkway of a street. Three plots of each treatment were on Pride of Mobile variety and one upon Daphne Pink. Four hundred flowers were counted on each plot. Phygon XL gave control at a concentration of 1 lb. per 100 gal., but gave less control as the concentration was reduced.

In 1949, Parzate Liquid compared favorably with Dithane D-14. Carbide and Carbon No. 658 and Bloomocide did not give control. Bloomocide produced some injury in the form of spotting of the flowers. In two other 1949 tests the antibiotic actidione, 5 p.p.m., did not give control.

Concentration of Effective Fungicides

A reduction in the concentration of the three most satisfactory materials, Dithane D-14, Dithane Z-78, and Parzate, was considered desirable to reduce spray residue and the possibility of injury. Accordingly, in 1948 and 1949 three concentrations of each of these materials were tested. The highest concentrations were: (a) Dithane D-14, 1⅓ qt.; zinc sulfate, 1 lb.; lime, ½ lb.; Triton B-1956, 1 fluid oz.; water, 100 gal.; (b) Dithane Z-78, 1.44 lb.; water, 100 gal.; (c) Parzate, 1.31 lb.; water, 100 gal. The medium concentrations were approximately 18 per cent below these and the lowest approximately 18 per cent below the medium (Table 2). The results of one of the two tests made each year are given in table 2. The location and arrangement of plots and the varieties used were the same as in the parkway tests described.

TABLE 2.—*Comparison of varying concentrations of Dithane D-14, Dithane Z-78, and Parzate sprays and of Dithane Z-78 and Parzate dusts in azalea petal blight control, 1948–1949*

Treatment: material and quantity used in 100 gal. water, or percentage as dust	Mean number diseased flowers per plot[a]		
	1948	1949	Mean
Dithane D-14, 1⅓ qt.; zinc sulfate, 1 lb.; lime, ½ lb.; Triton B-1956, 1 fl. oz.	5.0	108.75	56.9
Dithane D-14, 1.09 qt.; zinc sulfate, 0.82 lb.; lime, 0.41 lb.; Triton B-1956, 1 fl. oz.	24.5	133.5	79.1
Dithane D-14, 0.9 qt.; zinc sulfate, 0.67 lb.; lime, 0.34 lb.; Triton B-1956, 1 fl. oz.	14.5	172.0	92.8
Dithane Z-78, 1.44 lb.	48.5	184.75	116.8
Dithane Z-78, 1.18 lb.	47.25	261.75	154.5
Dithane Z-78, 0.97 lb.	80.5	272.5	175.3
Parzate, 1.31 lb.	27.0	202.0	113.3
Parzate, 1.08 lb.	4.0	231.25	117.6
Parzate, 0.88 lb.	9.5	235.25	119.9
Parzate, 6 per cent dust	39.0	270.5	154.8
Dithane Z-78, 6 per cent dust	49.0	315.5	182.3
Untreated	363.0	365.5	364.3
Least difference between means for significance			
19-1	72.6	121.2	62.0
99-1	96.9	162.1	84.2

[a] 400 flowers counted in each of 4 plots for each treatment.

Each of three concentrations of each material gave satisfactory control in 1948. There was a trend in this and in the second 1948 test (data not shown) for Dithane Z-78 to give the least control at 0.97 lb. to 100 gal. Control was less satisfactory in 1949. When compared with Dithane D-14, Dithane Z-78 and Parzate gave poorer control of the disease than in the previous year. This was more marked at the lower concentrations.

Wetting Agents

Santomerse S, 50 ml., and Dreft, 4 oz. to 100 gal., were used as wetting agents in comparison with Triton B-1956 in 1947. Treatments were repli-

cated four times in the parkway planting. Each treatment was applied to three plots of Pride of Mobile variety and to one plot of Daphne Pink. Four hundred flowers were counted on each plot. The data in table 3 show

TABLE 3.—*Control of azalea petal blight with Dithane D-14 + zinc sulfate + lime supplemented with different wetting agents, in 1947*

Treatment	Mean number of diseased flowers[a]
Dithane D-14, 1½ qt.; zinc sulfate, 1 lb.; lime, ½ lb.; Triton B-1956, 1 fl. oz.; water, 100 gal.	13.0
Dithane D-14, 1½ qt.; zinc sulfate, 1 lb.; lime, ½ lb.; Santomerse S, 2 fl. oz.; water, 100 gal.	2.0
Dithane D-14, 1½ qt.; zinc sulfate, 1 lb.; lime, ½ lb.; Dreft, 4 oz.; water, 100 gal.	6.8
Untreated	355.8
Least difference between means necessary for significance	
19-1	56.1
99-1	79.0

[a] 400 flowers counted in each of 4 plots per treatment.

that either Santomerse S or Dreft can be substituted for Triton B-1956 in preparing the Dithane D-14 spray.

Frequency of Application

Three applications a week were recommended by Westcott (11). Frequent applications have been considered necessary because of the continuous opening of the flowers on a plant over a period of 3 weeks or more. To determine whether three applications were necessary, three, two, and one applications a week of the Dithane D-14 spray were made during the 1946 to 1949 seasons along the parkway. Each treatment was replicated four times. In 1946 the treatments were randomized among Daphne Salmon, Daphne Pink, and Pride of Mobile varieties. In 1947 two plots were of Daphne Salmon variety and two of Pride of Mobile. In 1948 and 1949 three plots were of Daphne Salmon and one of Pride of Mobile. Four hundred flowers were counted on each plot each year. The 4-years' results are given in table 4. Spraying three times a week gave consistently better results than spraying twice. One application a week was unsatisfactory.

Comparable results were secured in 1946 and 1947 when one, two, and three applications of Phygon a week were made in tests arranged as the 1946 and 1947 Dithane D-14 tests. A summary of these data is given in table 4.

Spray Injury

Certain growers reported injury in 1946 following the use of the Dithane D-14 spray. Some were found to be using zinc sulfate containing 36 per cent metallic zinc and others the 25 per cent grade of zinc sulfate in preparing the spray. Westcott (11) used the latter. In 1947, sprays prepared with equal weights of zinc sulfate containing 25 and 36 per cent zinc were

TABLE 4.—*Comparison of 1, 2, and 3 applications a week of Dithane D-14 and Phygon sprays for control of azalea petal blight disease, 1946–1949*

Treatment	No. of weekly applications	Mean number of diseased flowers per plot[a]				
		1946	1947	1948	1949	Mean
Dithane D-14	3	10.5	6.5	10.8	81.8	27.4
Dithane D-14	2	27.8	46.8	57.6	176.8	77.3
Dithane D-14	1	232.3	151.3	96.3	283.3	185.8
Untreated		396.3	292.8	291.0	326.3	326.7
Least difference between means for significance						
19-1		89.2	128.1	116.1	92.1	48.7
99-1		128.1	184.1	166.8	132.3	65.8
Phygon	3	34.8	17.0			25.9
Phygon	2	103.0	43.8			73.4
Phygon	1	283.8	225.0			254.4
Untreated		396.3	292.8			344.5
Least difference between means for significance						
19-1		98.6	117.7			91.9
99-1		141.7	169.2			132.0

[a] 400 flowers counted in each of four plots for each treatment and year.

compared on plants growing in pots and under garden conditions. Injury resulted from the use of sprays containing the 36 per cent zinc sulfate under conditions which tended to dry out the flowers. A reduction in the amount of 36 per cent zinc sulfate reduced the injury. If the flowers were deficient in water for a longer period, injury was produced with any of the zinc sulfate concentrations in the Dithane sprays. A slightly greater water deficiency resulted in injury to unsprayed flowers. The spray injury consisted of a burning and drying of the petals from the margins inward. Because the injury was correlated with the water content of the flowers, sprayed plants should be watered during dry periods. Flower counts showed that varying the zinc sulfate concentrations had no effect on the control of the disease.

In 1947 the use of Phygon resulted in objectionable injury (2). On colored flowers this injury appeared first as a white stippling, followed by bleaching of the flowers and burning from the margins inward. The petals of white flowers became discolored and burned. Maximum flower injury resulted from three or more applications. Injury also occurred in 1948 following the use of Phygon or Phygon XL. Phygon XL was used at the rates of 1, ¾, and ½ lb. to 100 gal. water (Table 1). All concentrations produced injury, but the degree was lessened as the concentration was reduced.

Dusts

Dusts were tested because they might be applied with ease, particularly in small gardens. In 1947, a 10 per cent Fermate dust and a 0.5 per cent Phygon dust used on a parkway planting did not give favorable results.

Parzate and Dithane Z-78 in 6 per cent dusts were used in 1948 and 1949. The data (Table 2) show effective control with both dusts in 1948, whereas very poor control was obtained in 1949. In both these years the data cited were supported by another test giving similar results.

DISCUSSION

The control obtained in all 1949 tests was less than in previous years. This is thought to have been caused by the relatively high temperatures prevailing through the winter, which resulted in an irregular and early spring and in early blooming. These weather conditions also favored development of the petal-blight fungus, which attacked the early blooms. Since these early scattered blooms were not killed by cold, the disease continued to develop so that inoculum was plentiful when most of the flowers began to open and spraying was started. The disease was seen in two places on December 22, 1948, and appeared to have been present in one of these for at least a week. In the two preceding seasons the disease was first seen on December 30, but was held in check by later cold which killed the early flowers. One 1949 application was missed because of 2 days of continuous rainfall.

Dithane D-14, even at the lowest concentration used (Table 2), was more effective in 1949 than Dithane Z-78 or Parzate. This concentration was Dithane D-14, 0.9 qt.; zinc sulfate, 0.67 lb.; lime, 0.34 lb.; Triton B-1956, 1 fluid oz.; water, 100 gal. Although the Dithane Z-78 and Parzate were less satisfactory in these tests than Dithane D-14, it is believed that under ordinary conditions Dithane Z-78, 1.25 lb. to 100 gal., and Parzate, 1 lb. to 100 gal., can be safely recommended. They have the advantage over Dithane D-14 of requiring the use of only one material in the spray mixture. One grower who used Parzate in 1949 was more pleased with it than he had been with Dithane D-14 in previous years.

Six per cent dusts of Dithane Z-78 and of Parzate are believed worthy of recommendation to small gardeners because they can conveniently be used where only a few azaleas are to be protected. Spraying is the more feasible method in larger gardens and parkways because it affords better coverage than dusting.

Three applications of spray each week have consistently given better results than two, except during periods of dry weather when dews and fogs were lacking and few sources of infection were present. During dry weather the interval between applications may be increased provided a careful check is kept on the disease and the conditions favoring it. In dry periods this procedure would reduce some of the injury. Residue and injury are of special importance in spraying the flowers of ornamental plants. All spray materials used produced some residue; but by holding run-off to a minimum and using a fine spray, residue can be held to a minimum. The clumps of Parzate deposited on flowers and foliage were not objectionable, but an accumulation of dusts on the flowers might be.

SUMMARY

Results of 4 years' tests for control of Azalea petal blight (*Ovulinia azaleae* Weiss) are given. A number of new fungicides were tested. The Dithane D-14 spray mixture previously recommended (11) has proved effective, as have Dithane Z-78, Parzate, and Parzate Liquid. Reduced concentrations of the Dithane D-14 spray, the Dithane Z-78, and the Parzate have afforded satisfactory control.

Either Santomerse S or Dreft can be satisfactorily substituted for Triton B-1956 as wetting agents in the Dithane D-14 spray.

Dithane D-14 sprays prepared with the same weight of zinc sulfate containing either 36 per cent or 25 per cent metallic zinc were compared. Injury was more likely with higher concentration of zinc under conditions which dried out the flowers. Control was not affected by this difference in zinc content.

Phygon, previously recommended (11), and Phygon XL controlled the disease but produced objectionable injury.

Three applications a week of Dithane D-14 or Phygon have afforded better control than one or two applications.

Parzate and Dithane Z-78 dusts offer reasonably good control and are convenient for the small gardener to use.

U. S. DEPARTMENT OF AGRICULTURE
SPRING HILL, ALABAMA

LITERATURE CITED

1. BRIERLEY, PHILIP. Azalea flower spot (Ovulinia azaleae) appears in Maryland. U. S. Dept. Agr., Pl. Dis. Rptr. **30**: 244–245. 1946.
2. GILL, D. L. Azalea petal blight control brought up to date. Home Gardening for the South **8**: 19–21. 1948. (*Also in* Sou. Flor. and Nurseryman **60**(41): 9–10. 1948.)
3. ————. Current information on azalea petal blight and its control. Home Gardening for the South **8**: 270–271, 284–285. 1948. (*Also in* Flor. Exch. **111**(21): 14–15. 1948; Flor. Rev. **103**(2660): 38–39, 102–103. 1948; *and* Sou. Flor. and Nurseryman **61**(34): 14–15, 53–55. 1948.)
4. MILBRATH, D. G. Occurrence of azalea flower spot in California. U. S. Dept. Agr., Pl. Dis. Rptr. **25**: 318. 1941.
5. NUGENT, T. J. Azalea petal blight in the Norfolk area of Virginia. U. S. Dept. Agr., Pl. Dis. Rptr. **31**: 244. 1947.
6. PLAKIDAS, A. G. Effect of sclerotial development on incidence of azalea flower blight. U. S. Dept. Agr., Pl. Dis. Rptr. **33**: 272–273. 1949.
7. WEISS, FREEMAN. A fungus spot of azalea flowers. (Abstr.) Phytopath. **25**: 38. 1935.
8. ————. Ovulinia, a new generic segregate from Sclerotinia. Phytopath. **30**: 236–244. 1940.
9. ————, and FLOYD F. SMITH. Present status of azalea flower spot. (Abstr.) Phytopath. **28**: 21. 1938.
10. ————, and ————. A flower spot disease of cultivated azaleas. U. S. Dept. Agr. Circ. 556. 1940.
11. WESTCOTT, CYNTHIA. Azalea petal blight can be controlled. Home Gardening for the South **5**: 134–139. 1945.

PHYSIOLOGY OF TOXIN PRODUCTION BY CERATOSTOMELLA ULMI[1]

A. W. Feldman, N. E. Caroselli, and F. L. Howard[2]

(Accepted for publication September 6, 1949)

Investigations have shown (7, 8, 9) that *Ceratostomella ulmi* produces a toxic substance in culture which, when injected into elms, is capable of causing symptoms typical of the Dutch elm disease. Two toxic substances obtained from culture filtrates were identified by Dimond (2) as a polysaccharide and an alcohol-soluble component. Each fraction produced symptoms typical of a part of the syndrome of Dutch elm disease; *i.e.*, the polysaccharide produced an upcurling of the leaves with marginal withering; the alcohol-soluble fraction produced interveinal necrosis.

Investigations have shown (1, 6) that the toxin responsible for the wilting of test plants could also be used for assaying toxin-neutralizing chemicals. When cuttings of snapdragon, bean, eggplant, tomato, elm, and maple were placed in tubes containing sterile filtrates from cultures of the fungus, wilting resulted in 24 to 48 hr. The ultimate goal of the present investigation has been to find therapeutic chemicals that could be used to prevent or reduce the destructive effects of the Dutch elm disease.

Before any chemicals could be screened, a knowledge of some of the factors influencing toxin production was needed in order to delineate a reference point for standardization in assay as well as to elucidate some of the physiological capabilities of the organism.

MATERIALS AND METHODS

Two markedly pathogenic isolates of *Ceratostomella ulmi*, a "black" strain and OS89845, supplied by Dr. O. N. Liming, were tested. Unless otherwise stated, data given were based on the black strain because of its greater toxin-producing power.

A liquid synthetic medium modified somewhat for the buffer series was used in all tests. Its composition was: glucose, 25 gm.; l-asparagine, 2 gm.; KH_2PO_4, 1.5 gm.; yeast extract, 2 gm.; $MgSO_4 \cdot 7\ H_2O$, 1 gm.; $FeCl_3$, 0.010 gm.; H_2O, 1000 cc. (7). Fifty cc. were dispensed into 250-cc. Erlenmeyer flasks and autoclaved 15 min. at 15 lb. pressure. The pH was 5.25 after sterilization.

The inoculum was maintained on a 1.5 per cent agar medium having the same composition as that of the liquid medium. After the fungus had grown for 2 weeks at 26° C., uniform disks were removed and used to inoculate each flask of liquid medium. Then the flasks were put in a shake

[1] Contribution No. 748 of the Rhode Island Agricultural Experiment Station.

[2] Assistant research professor, R.I.S.C.; associate pathologist, Bartlett Tree Research Laboratory; and research professor, R.I.S.C., respectively. The technical assistance of Mary-Elizabeth Davies is gratefully acknowledged.

machine at 26° C. and agitated by 58 4-in. strokes per min. From the second through the seventh or ninth day, 2 flasks were removed daily from the shake table, filtered, and assayed for toxin, and the composition of the medium was analyzed. All experiments were repeated at least twice.

Three-weeks-old, sand-grown Pennhart tomato plants were used to evaluate the potency of the toxin. These plants were reasonably uniform, succulent, and sensitive to the toxin.

The procedure for determining the potency of the toxin was as follows: The mycelium was separated from the medium by filtration and the filtrate was then sterilized by passage through sintered glass. One cc. of the sterile filtrate was put into each of 10 ligature tubes 9 × 30 mm. Stems of the tomato plants were cut under water at an angle, with a sharp scalpel, and one plant was inserted into each tube; the plants were kept at 26° C. and at a relative humidity of 80 per cent. Data were recorded on the amount and type of wilting, *i.e.*, stem or leaf or both (total), at the end of 6, 12, and 24 hr. Water and noninoculated medium checks were run with each test. Before assay, the pH of the filtrate was always adjusted to that of the original medium.

The use of small, uniform plants and small amounts of liquid made it unnecessary to measure the liquid uptake or the percentage of leaf surface affected. Potency of the toxin was not materially affected by storage. Therefore, the culture filtrates were stored at 2° C. and assayed later when it was convenient to make all assays on the same day. Results for a given test, when repeated a month later with the same refrigerated filtrate, varied no more than 10 per cent.

RELATION OF POLYSACCHARIDE FRACTION TO WILTING

Since the polysaccharide fraction was shown to be capable of producing wilt symptoms (2), it was believed that this fraction might be used as a criterion to standardize the toxin titer. Therefore, in order to learn some of the changes taking place in the medium during the course of polysaccharide formation and to determine, if possible, the factors influencing the initiation of polysaccharide production, the following data were taken: Mycelial weight (dry), pH, acid production, reducing substances, polysaccharide yield, and wilt index produced by the culture filtrate.

The polysaccharide fraction was isolated from a 50-cc. aliquot of the combined filtrate from 2 flasks after the pH was adjusted to that of the check (5.25). Fractionation was carried out at 30° C. *in vacuo*. After the volume was reduced to 10 cc., 28 cc. of 95 per cent ethanol was slowly added while the mixture was kept in constant agitation. The crystalline polysaccharide usually precipitated immediately and was removed by centrifuging after the mixture remained for 24 hr. at 0° C.

Mycelial weight, pH, and wilt index were determined for each flask. Total acid production, expressed as a cc. equivalent, was calculated on a combined 25-cc. aliquot by titrating to the pH of the check (5.25) with

N/100 NaOH. Total reducing substances were determined by the Hagedorn-Jensen method (4) and were run in triplicate for each flask culture

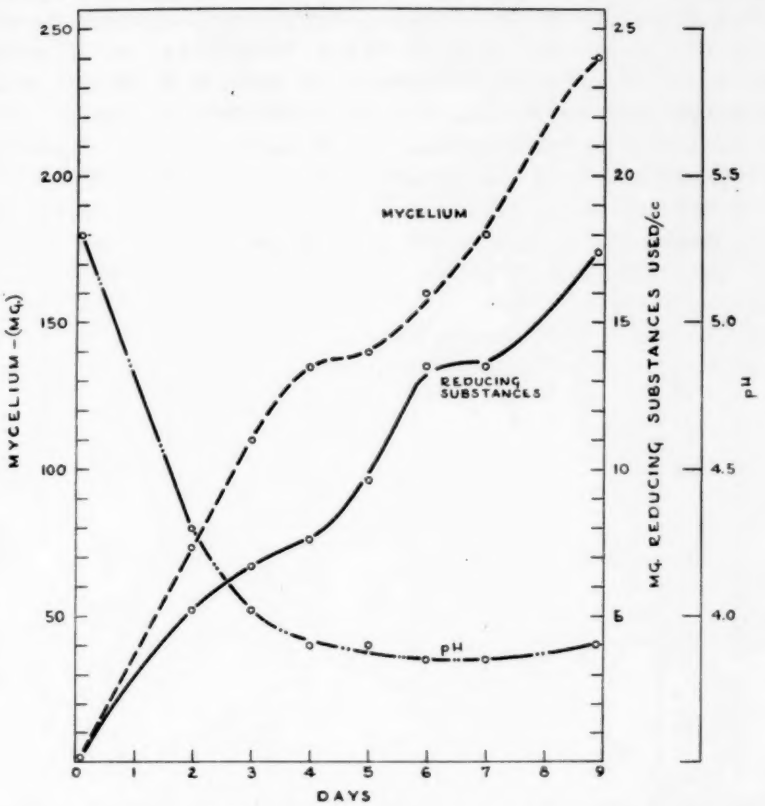

FIG. 1. Relationship of the growth of *Ceratostomella ulmi* (dry weight of mycelium) to reducing substance utilization and pH changes occurring in a weakly buffered medium at 26° C.

Rate of growth, utilization of reducing substances, and pH are shown in figure 1. The rate of reducing substance utilization was found to parallel the growth rate of the fungus. In every case the pH dropped rapidly to

TABLE 1.—*The relation of the amount of acid produced by Ceratostomella ulmi in culture each day to toxin produced as evidenced by the percentage of tomato seedlings wilting within 24 hr.*

Days	Acid produced (cc. equiv.)	Total wilt (per cent)
2	4.8	10
3	7.3	10
4	10.4	20
5	10.2	80
6	15.1	60
7	13.0	90
9	11.8	100

4.0 by the third or fourth day and then remained relatively constant. There was no correlation between the amount of acid produced by the organism and the amount of wilt caused by the same culture filtrate when assayed (Table 1). For example, 10.4 cc. of acid equivalent were produced on the fourth day with a resultant wilt index of 20 per cent, and 10.2 cc. acid equivalent on the fifth day with a resultant wilt index of 80 per cent, although the pH was the same on both days. The rate of growth and reducing substance utilization likewise bore no relationship to the wilt index. When data on the polysaccharide yield were plotted against the wilt indices obtained at daily intervals with the culture filtrates (Fig. 2), no direct correlation between polysaccharide formation and wilting was apparent.

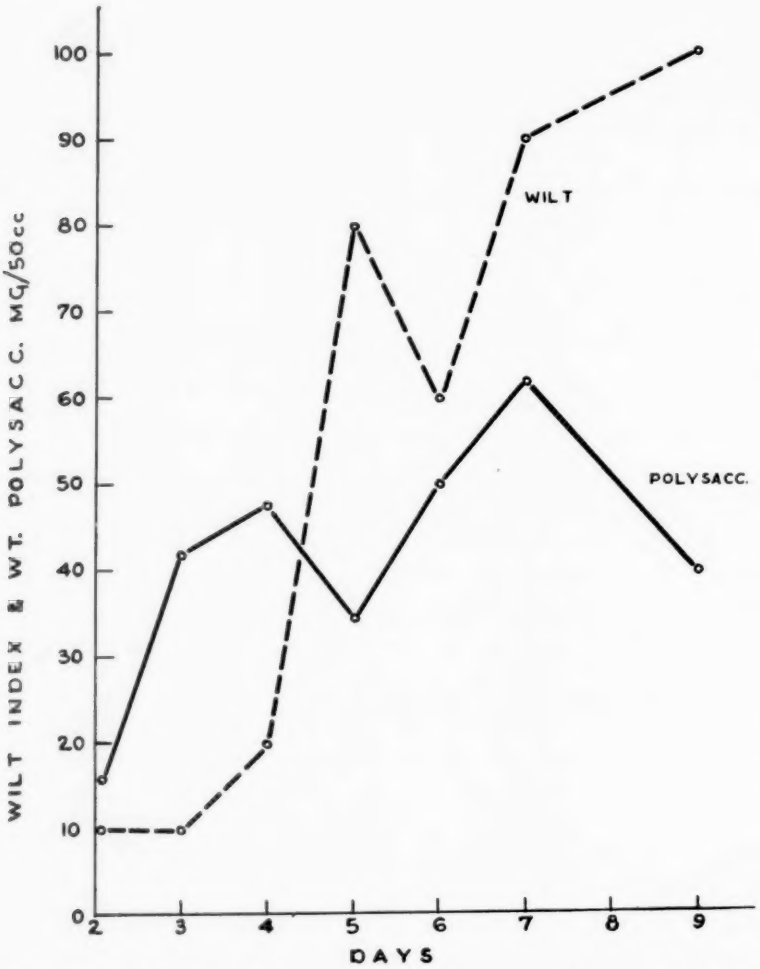

FIG. 2. Relation between the weight of the polysaccharide present in the culture filtrate and tomato wilt index shows no direct correlation between polysaccharide formation and wilting.

The toxic metabolite always appeared within 24 to 48 hr. after the pH reached 4.0. Since the pH of the filtrate was adjusted before assay to that of the original medium, the wilt indices were not a result of the pH changes. According to this evidence, the role of the polysaccharide as a toxic component seemed obscure. Therefore, to determine whether or not this fraction was responsible for wilting, the following experiments were performed: Approximately 1.95 gm. of polysaccharide was isolated from 32 flasks of a 7-day shake culture of the organism. The polysaccharide was purified once by dissolving it in a small quantity of distilled water and reprecipitating it with 95 per cent ethanol. After it was dried at 50° C., the polysaccharide was added to unused medium in concentrations of 0.5 to 4 mg. per cc. in increments of 0.5 mg. Ten plants were tested at each concentration. Data on the percentage of wilt obtained for each concentration of the polysaccharide are shown in figure 3. No reasonable explanation can be given for the

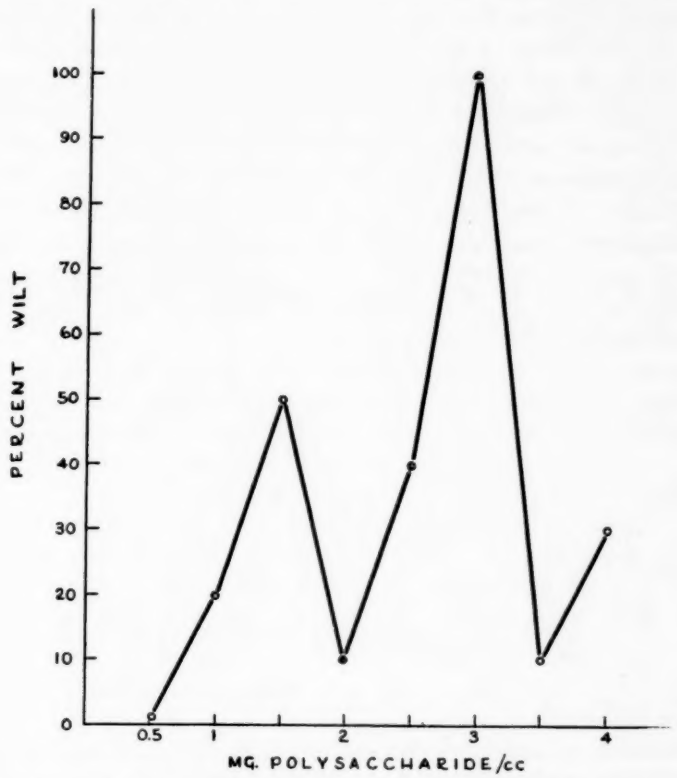

FIG. 3. Per cent wilt obtained with various concentrations of the pure polysaccharide added to unused medium after 24 hr. Assay performed with 3-weeks-old, sand-grown tomato plants at 26° C.

results; wilt indices of 50 and 100 per cent were obtained with 1.5 and 3 mg. polysaccharide per cc., respectively, while only 10 per cent wilt was observed at the polysaccharide concentrations of 2 and 3.5 mg. per cc.

The maximum concentration of polysaccharide obtained from a culture filtrate with a wilt index of 100 per cent was 1.2 to 1.3 mg. per cc. This amount of polysaccharide would have a wilt index of 30 to 40 per cent, if the wilting of the test plants was caused by the polysaccharide. The type of wilt symptom caused by the polysaccharide was similar to that described by Dimond (2).

FRACTIONATION OF THE CULTURE FILTRATE

The wilt-inducing properties of the polysaccharide in the culture filtrate were investigated in the following manner: First, the alcohol-soluble fraction was assayed after the polysaccharide was removed; second, the polysaccharide was assayed alone, as well as when recombined with the alcohol-soluble fraction. The culture filtrate was a reference standard in the assay. A 50-cc. aliquot of the culture filtrate was used for fractionation. The procedure for isolating the polysaccharide was the same as that outlined above. After the polysaccharide was removed, the ethanol in the residue (alcohol-soluble fraction) was removed by distillation *in vacuo* at 30° C. Volume was returned to the original 50 cc. by adding distilled water. For assay, one-half of each day's yield of polysaccharide was dissolved in 25 cc. of unused medium, and the other half was dissolved in 25 cc. of the alcohol-soluble fraction of the corresponding day. Data on the percentage of wilt obtained with the culture filtrate from the third through the eighth day are shown in table 2. These are compared to the percentage

TABLE 2.—*Percentage of wilt obtained with the original culture filtrate of Ceratostomella ulmi, polysaccharide alone, alcohol-soluble fraction alone, and polysaccharide plus alcohol-soluble fraction*[a]

Days after inoculation	Culture filtrate[b]	Polysaccharide fraction[c]	Alcohol-soluble fraction[b]	Polysaccharide plus alcohol-soluble fraction[b]
3	0	30	0	0
4	10	30	0	0
5	30	50	10	0
6	80	20	70	100
7	100	20	100	90
8	40	10	70	100

[a] These data were obtained with isolate OS89845; comparable results were obtained with the "black" strain.
[b] Total wilt after 24 hr.
[c] Leaf wilt after 24 hr.

of wilt obtained with the polysaccharide alone, polysaccharide plus alcohol-soluble fraction, and alcohol-soluble fraction alone.

Comparison of the wilt data of the alcohol-soluble fraction with that of the original toxin shows that the polysaccharide contributed very little to the amount of wilt. Further evidence of this trend is obtained by comparing the wilt data on the alcohol-soluble fraction alone and on the combination with the polysaccharide. Only leaf wilt was observed with the poly-

saccharide. In every case wilt was slight when compared to the original culture filtrate or to the series containing the alcohol-soluble fraction. Total wilt was always observed with the culture filtrate and the series containing the alcohol-soluble fraction.

These data show that the polysaccharide fraction cannot be used as a reference point for toxin standardization.

EFFECT OF pH OF THE MEDIUM ON TOXIN PRODUCTION

In previous experiments on the relationship of various factors to toxin production, a comparatively high titer of toxin appeared after the pH of the medium reached the proximity of 4.0. This constant association suggested the possibility of the toxin being produced either by a response to successive pH changes or to a specific pH range. Since the initial pH of the medium was 5.25 and toxin production increased as pH 4.0 was approached, two pH levels were tested. These were 4.25 and 5.25. To prevent any pH fluctuations, the medium was buffered with a citric acid + NaOH – HCl solution (3). The basal medium was that used in the previous experiments. For pH 5.25, 250 cc. of citric acid + NaOH were added to the medium to make 1 liter, and 250 cc. of citric acid + HCl for pH 4.25.

Data were taken on mycelial weight, pH, wilt index, and polysaccharide yield from the third through the seventh day. Slight pH changes were observed daily in both buffer series. In the series buffered at pH 5.25, the pH reached 4.75 on the fifth day and then remained constant. In the pH 4.25 series, the pH finally reached 4.10 on the seventh day. Growth was better in the less acid medium, but toxin was produced more rapidly and with a higher titer in the medium buffered at pH 4.25 (Fig. 4). A constant wilt index of 100 per cent (except on the sixth day) was observed from the fourth through the seventh day with the pH 4.25 buffered medium. In the series buffered at pH 5.25 the wilt index slowly increased until it reached a maximum of 60 per cent on the seventh day.

The same buffered cultures were tested again, and information was taken on the third through the 13th day. Data for wilt indices with the pH 4.25 buffer were the same as noted above. On the 13th day the pH was 4.25; mycelial weight was 200.2 mg. A maximum wilt index of 80 per cent was obtained on the 13th day with the cultures buffered at 5.25. The pH had returned to 5.0 and the mycelial weight was 205.9 mg.

The same basal medium, buffered at pH 7.0 with 250 cc. citric acid and N/5 NaOH, was used in another test. The buffer capacity was apparently not sufficient to prevent a change in pH. By the seventh day, the pH had decreased to 5.1 and the wilt index had risen to 30 per cent after remaining at a 10 per cent level. The mycelial weight on the seventh day was 171.6 mg. as compared to 146 mg. for the corresponding day with the series buffered at pH 4.25.

In no case could polysaccharide be extracted from any of the buffered series.

These data indicate that successive pH changes are not necessary for toxin production. Some change in pH was manifested with the series buffered at pH 7.0, but no appreciable change was noted even after 13 days in the series buffered at pH 4.25 and 5.25. Data from the series buffered at pH 4.25 seem to demonstrate that for optimum toxin production the specific pH range is 4.0 to 4.25. Furthermore, the rate of mycelial growth bears no direct relation to toxin production.

A comparison was made of the tomato seedling wilt symptoms caused by culture filtrates from the series buffered at both pH 4.25 and pH 5.25. Although a wilt index of 60 per cent was obtained on the third day with the pH 4.25 culture filtrate and on the seventh day with the pH 5.25 cul-

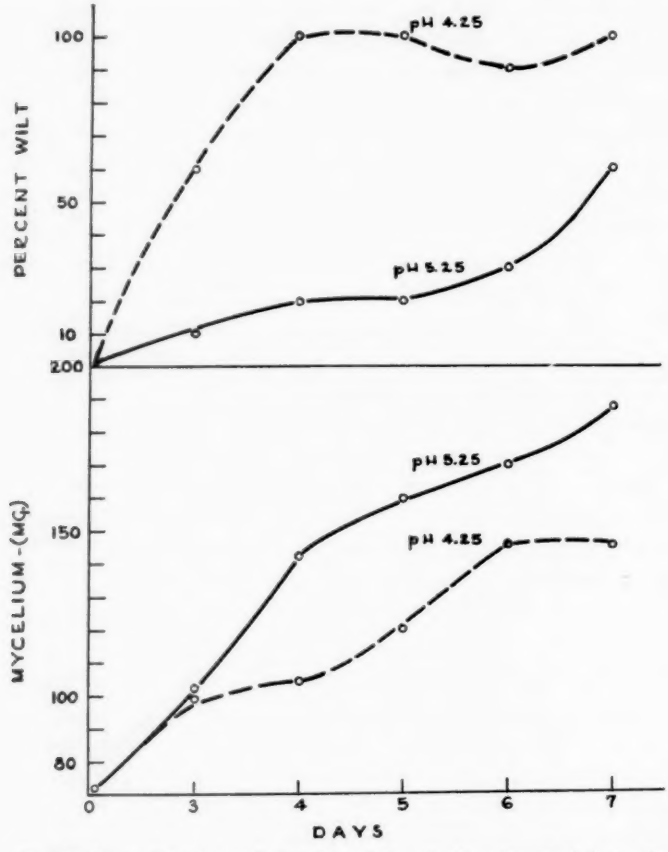

FIG. 4. Production of toxin and growth of *Ceratostomella ulmi* in buffered media.

ture filtrate, there was a difference in degree and severity of the wilt symptoms noted after 24 hr. exposure. All seedlings in the pH 4.25 culture filtrate had flaccidity of the leaves and stems on the third day; complete collapse of the stems was noted in 60 per cent of the plants. The symptoms usually developed rapidly. During the first 6 hr. the leaves lost some of their

turgidity and showed a slight downward rolling of the margins. After 12 hr., flaccidity was apparent in the stems and petioles and some had totally wilted. The seedlings had no downward rolling of leaf margins until after 12 hr. in filtrates from 7-day cultures at pH 5.25. No flaccidity of the stems and petioles was apparent then. After 24 hr., 60 per cent of the stems were flaccid but not collapsed. The leaves of these plants were partly flaccid but there was no constriction of the petioles. Although these plants were totally wilted, the wilt symptoms were never so severe as those observed with a 3-day culture filtrate from the series buffered at pH 4.25.

The titer of the toxin obtained on the fourth through seventh days from the pH 4.25 culture filtrates was reflected in the degree of wilting observed at a specific time. In general, from a 7-day pH 4.25 culture filtrate, total wilt of all the plants was observed within 12 to 18 hr. After 24 hr. these plants were completely withered and their condition reflected the degree of toxin potency. Although the data on wilt indices for the pH 4.25 culture filtrate series, as seen in figure 4, show 100 per cent wilt for the fourth, fifth, and seventh days, the same toxin titer was not present in each series. For the sake of simplicity these data were plotted from the wilt indices obtained after 24 hr. exposure. Had the data been plotted for the 36-hr. period, all series would have shown 100 per cent wilt with the pH 4.25 culture filtrate. After 48 hr. those plants in the 7-day pH 5.25 culture filtrate were 100 per cent wilted.

Although similar results were obtained with isolate OS89845 at the various pH levels tested, the titer of the toxin decreased after the seventh or ninth day in the series buffered at pH 5.25.

RELATIONSHIP OF CULTURE FILTRATE PH TO WILTING

According to the data presented above, there is a direct correlation between the pH of the medium in which the organism is grown and the potency of the toxin in the culture filtrate. Since it appeared that the toxin was more rapidly produced at a specific pH level, the question was raised whether the pH of the medium also affected the toxin itself.

The initial tests to determine the effect of pH on the toxin were run with the pH 4.25 buffered medium because the fungus produced a consistently high titer of toxin within 7 days. Approximately 700 cc. of culture filtrate were taken from 15 flasks. After initial filtration to remove the mycelium, the filtrate was sterilized by passage through sintered glass and then divided into 2 lots each containing 350 cc. In the first lot separate 50-cc. aliquots were adjusted for pH 4.0 through 8.0. Since the filtrate was pH 4.05, this was used unaltered for the pH 4.0 series. The remainder was adjusted with N/NaOH to the pH desired. There was insignificant dilution of the toxin since 1 cc. was the maximum base added. A corresponding pH series of unused medium served as a control.

The same procedure was followed for the second lot of culture filtrate, except that after the pH series was prepared it was heated to 100° C. and

cooled immediately. A similar pH series was made with unused medium and heated in the same manner. Table 3 shows data on the percentage of wilt obtained with both the heated and unheated lots of each pH series.

The toxin is apparently inactivated at pH 6.0 and above for both the heated and unheated series. Heating caused some inactivation of the toxin

TABLE 3.—*Percentage of total wilt observed 24 hr. after adjusting the pH and heating the filtrate of a 7-day culture*

Adjusted pH of filtrate	Percentage of total wilt	
	Unheated	Heated
4[a]	100	70
5	80	40[b]
6	30	40
7	20	20
8	10	10

[a] Unadjusted, since the pH of the filtrate of a 7-day culture was 4.05.

[b] The percentage of wilt was variable. Greatest amount of wilt observed was 90 per cent. No wilt was observed for any of the pH series (heated or unheated) with the unused medium.

at pH 4.0 and possibly at 5.0, but not at the other pH levels tested. No wilt occurred in either the heated or the unheated series of unused medium at the various pH levels.

To determine whether the reduction of the wilt indices at pH 6.0, 7.0, and 8.0 was a result of toxin inactivation rather than a decrease in cellular permeability of the test plants to the toxic metabolite at these pH levels, the following experiments were carried out. Aliquots of a 7-day culture filtrate were adjusted to pH 6.0, 7.0, and 8.0 with N/NaOH. After 2, 4, 24, and 72 hr., 25-cc. aliquots of each of the three pH levels were readjusted to the original pH of the culture filtrate (4.09) with N/HCl. A

TABLE 4.—*Wilt indices obtained by adjusting the pH of a 7-day culture filtrate to pH 6.0, 7.0, and 8.0, then readjusting the pH 2 hr. later to that of the original culture filtrate (pH 4.09)*

Filtrate pH adjusted to	pH readjusted after 2 hr. to	Percentage of total wilt, 24 hr.[a]
4.09	4.09	100
6.0	4.09	30
7.0	4.09	20
8.0	4.09	10

[a] No wilt was observed in the control series with unused medium treated in the same manner.

corresponding control series with unused medium was also tested. As table 4 shows, adjusting the pH of the culture filtrate to 8.0 and readjusting it to its original pH (4.09) after 2 hr. did not restore the activity of the toxin. A comparison of tables 3 and 4 indicates that the wilt indices of the culture filtrate adjusted to pH 6.0, 7.0, and 8.0 remained unchanged

after readjustment to their original pH. After 4, 24, and 72 hr., when the pH was readjusted to the original level, the results were comparable to those of the 2-hr. series. Two additional experiments were made. In one, the pH was adjusted with N/KOH and in the other with $Ca(OH)_2$ (saturated solution). The same pH levels were tested and the pH was readjusted to that of the original culture filtrate after 2, 4, and 24 hr. with N/HCl. Results obtained with KOH as the base were comparable to those obtained with NaOH. No wilting was observed with the $Ca(OH)_2$ series at pH 6.0, 7.0, and 8.0 nor after these three pH levels were readjusted to the pH of the original culture filtrate. These lower wilt indices were probably due to dilution of the toxin, since fairly large quantities of $Ca(OH)_2$ were needed to attain the desired pH.

A comparison of effect of the cation of NaOH and KOH on the heated toxin was made. A series adjusted to pH 8.0 with NaOH and another with KOH were heated to 100° C., immediately cooled and then readjusted to pH 4.10 after standing 2 hr. No difference in the degree of toxin inactivation could be observed between the sodium and potassium ions with the heated series. Both hydroxides equally inactivated the toxin, and readjusting the pH to 4.10 did not restore toxin activity.

Data from the preceding experiments indicate that the toxin in the culture filtrate is irreversibly inactivated by hydroxyl ions at pH 6.0 and above. Although toxin inactivation was greatest at pH 8.0, the degree of inactivation appeared to be independent of the associated cations. Accordingly, it is possible that cellular permeability of the test plants may have played some role to account for the differences in wilt indices observed at the various pH levels. However, the major effect can be attributed to toxin inactivation by hydroxyl ions rather than to a decrease in permeability of test plants to the toxin in the more alkaline pH ranges. The length of time required for the inactivation of the toxin by hydroxyl ions cannot be stated with certainty. The shortest period tested was 2 hr., but it is entirely possible that this inactivation could have taken place immediately upon the addition of the hydroxyl ions.

In view of the present data, the question is whether any toxin is produced at pH 7.0. Since the nature of the toxic complex has not been determined, despite numerous attempts, it is unknown whether small amounts of toxin are produced or whether larger amounts are produced but are inactivated at this pH. In those experiments in which the organism was grown in a medium buffered at pH 7.0, only small amounts of toxin were present even after the pH reached 5.10 on the seventh day. Assay was performed on the culture filtrates from this experiment after the pH was readjusted to 7.0. A maximum wilt index of 30 per cent was obtained for the culture filtrate on the seventh day. Similarly, but without readjusting the pH to 7.0 before assay, there was only a slight increase in the wilt index. This suggests that only small amounts of toxin are developed in the pH 7.0 culture filtrate and that increased hydrogen-ion content of the

medium is conducive to toxin production. Nevertheless, absolute proof is wanting regarding toxin formation in a neutral or slightly alkaline medium.

PROCEDURE FOR SCREENING THERAPEUTANTS

The information obtained on toxin production was used to develop standard bioassay techniques for toxin-antidoting chemicals. Since the toxin is most rapidly produced by the "black" strain of *Ceratostomella ulmi* in a medium buffered at pH 4.25 after 7 days, the filtrate from this strain was used for the assays. Dosages of 1–1,000, 1–5,000, and 1–10,000 of the test chemicals were added directly to the culture filtrate. Only 20 cc. of culture filtrate were needed for each concentration of the chemical used. Since a pH of 6.0 or above inactivates most of the toxin, pH determinations were made on the culture filtrate plus the test chemical. The buffer capacity of the filtrate was high enough generally to prevent change in the pH by the test chemical. A check series was run with the test chemicals added to unused buffered medium. Several times, the test chemical was first dissolved, either in 1 cc. of 95 per cent ethanol or in isopropanol, before being added to the culture filtrate. In such cases, additional checks were run with both the noninoculated medium and the culture filtrate plus alcohol. Wilt readings of the seedling tomato plants were taken after 24 and 48 hr. All culture filtrate checks caused complete wilting in 24 hr. Plants in the treated culture filtrate series showing no wilt, or partial wilt, after 24 hr. were allowed to remain for 48 hr. to determine whether additional wilting would occur. Chemicals found *per se* to be phytotoxic at the concentrations tested were retested in dilutions of 1–20,000, 1–50,000, and 1–100,000.

This procedure to evaluate chemicals for their toxin-antidoting properties has proved efficient for screening a large number of compounds. For example, of 250 organic and inorganic chemicals tested, 15 have been found capable of antidoting the toxin. These chemotherapeutants are now being further tested in the greenhouse on seedlings of *Ulmus americana* and in the field on large elms for therapeutic and/or prophylactic properties for combating the Dutch elm disease.

DISCUSSION

Previous studies have shown that polysaccharide and nonpolysaccharide substances such as soluble starch, crown-gall polysaccharide, *Ceratostomella ulmi* polysaccharide, polyethylene glycols, and polyvinyl alcohols are capable of inducing wilt in plants (2, 5). When the polysaccharide produced by *C. ulmi* was extracted from the culture filtrate, added to unused medium in concentrations of 0.5 to 4.0 mg. per cc., and tested for wilt-inducing properties, a wilting of the test plants resulted. The amount of wilt observed, however, was not consistent with the concentrations in the solution test (Fig. 3). The actual role of the polysaccharide relative to wilting is

obscure. Nonetheless, there is evidence to show that the polysaccharide cannot be considered the prime wilt-inducing component of the culture filtrate. The evidence for this statement is: (a) When the polysaccharide yield was plotted against the daily wilt indices obtained with the culture filtrate there was no direct correlation between polysaccharide formation and wilting (Fig. 2). (b) The maximum concentration of polysaccharide obtained from a culture filtrate having a wilt index of 100 per cent was 1.2 to 1.3 mg. per cc. This amount of polysaccharide added to noninoculated medium and assayed yielded a mean wilt index of 35 per cent. (c) Fractionation of the culture filtrate and assay of the components showed that the polysaccharide *per se* contributed very little to raise the wilt index of the polysaccharide plus alcohol-soluble fraction above that of the alcohol-soluble fraction alone. (d) A high titer of toxin was rapidly produced in a medium buffered at pH 4.25, yet no polysaccharide was produced. Similarly, it was found that the polysaccharide was not formed in cultures buffered at pH 5.25 and 7.0.

There is *a priori* evidence to suggest that the polysaccharide may not be produced in diseased trees; or, if produced, that it may not be responsible for the wilt symptoms. This is supported by the fact that basic chemicals used to inactivate the toxin *in vitro* are without effect on polysaccharide-induced wilting of tomato cuttings. Furthermore, introduction of a solution of $Ca(OH)_2$ into trees at the onset of wilt symptoms resulted in a cessation of wilting for a period of at least 3 weeks, while introduction of water into wilting trees generally retarded wilt symptoms for only 24 to 48 hr.

Practical application has been made of the information obtained from the effect of hydroxyl ions on toxin inactivation *in vitro* as well as on the effect of pH on toxin production. During the past 2 years through soil application of basic chemicals it has been possible not only to retard the destructive effects of the Dutch elm disease, in both greenhouse and field experiments, but also to increase the effectiveness of both therapeutic and prophylactic chemicals.

CONCLUSIONS

Procedures have been standardized for toxin production so that screening of toxin-neutralizing chemicals is now possible.

The relationship of such factors as mycelial weight, age, pH, acid production, reducing-substance utilization, and polysaccharide yield were studied on liquid shake cultures of *Ceratostomella ulmi* and were measured by wilt indices of sand-grown tomato cuttings.

The wilt indices of the culture filtrates bore no relationship to mycelial growth, acid production, reducing-substance utilization, or polysaccharide production.

In weakly buffered media, the fungus caused the pH to drop rapidly to 4.0 by the third or fourth day and then remain relatively constant. The toxin then appeared within 24 to 48 hr. after the pH reached 4.0. Frac-

tionation of this culture filtrate yielded a polysaccharide and an alcohol-soluble fraction.

Toxin with a high titer was rapidly produced in sodium citrate-HCl buffered media at pH 4.25. Less toxin was produced in media buffered at 5.25, while very little toxin was evident in media buffered at pH 7.0.

Polysaccharide formation was prevented in the culture filtrate by buffering with a citric acid-NaOH-HCl solution, but the toxic alcohol-soluble fraction was present.

Although pure polysaccharide can cause wilting of the tomato cuttings, there was no relationship between the concentration of the polysaccharide and its wilt index.

It is suggested that polysaccharide is not the prime toxic component produced by *C. ulmi*.

The toxin in the culture filtrate is irreversibly inactivated by hydroxyl ions, irrespective of the associated cations, at pH 6.0 and above.

RHODE ISLAND AGRICULTURAL EXPERIMENT STATION
KINGSTON, RHODE ISLAND
AND
BARTLETT TREE RESEARCH LABORATORY
STAMFORD, CONNECTICUT

LITERATURE CITED

1. CAROSELLI, N. E. Bleeding canker. M. S. thesis. R. I. State College. 1940.
2. DIMOND, ALBERT E. Symptoms of Dutch elm disease produced by *Graphium ulmi* in culture. (Abstr.) Phytopath. 37: 7. 1947.
3. GORTNER, R. A. Outlines of Biochemistry. 1017 pp. John Wiley & Sons, Inc. 1938.
4. HEINZE, P. H., and A. E. MURNEEK. Comparative accuracy and efficiency in determination of carbohydrates in plant material. Mo. Agr. Exp. Sta. Res. Bul. 314. 1940.
5. HODGSON, ROLAND, W. H. PETERSON, and A. J. RIKER. The toxicity of polysaccharide and other large molecules to tomato cuttings. Phytopath. 39: 47–62. 1949.
6. HORSFALL, J. G., and G. A. ZENTMYER. Antidoting the toxins of plant diseases. Phytopath. 32: 22–23. 1942.
7. ZENTMYER, G. A. Toxin formation and chemotherapy in relation to Dutch elm disease. (Abstr.) Phytopath. 32: 20. 1942.
8. ———, and J. G. HORSFALL. Toxin formation by *Ceratostomella ulmi*. Science 95: 512–513. 1942.
9. ———, ———, and P. P. WALLACE. Dutch elm disease and its chemotherapy. Conn. Agr. Exp. Sta. Bul. 498. 1946.

ROOT AND COLLAR WINTER INJURY OF APPLE TREES

J. S. Cooley and A. B. Groves[1]

(Accepted for publication October 11, 1949)

Studies on apple root rots have been carried on by the writers at intervals since 1932 (4). Although special attention was given to pathogenic root diseases in this work, it was noted that there were certain disorders that apparently could not be attributed directly to pathogens. Foremost among such nonpathogenic disorders were collar and root lesions that seemed to be caused by winter injury.

The literature on root and collar disturbances of apple trees comprises reports of work carried on in regions having various climatic conditions. In one locality the collar injuries may be attributed to environmental influences, whereas an investigator working where conditions were different may attribute the collar injuries there to a pathogen.

Grossenbacher (6), Thomas (11), Magness (7), and others working in the colder parts of the United States, particularly in New York and Washington States, have attributed the prevailing collar disturbances in those regions to winter injury.

Collar blight has been prevalent on Grimes Golden apple in Virginia and other regions where this variety is extensively grown. At one time the fire-blight organism (*Erwinia amylovora* (Bur.) Winslow *et al.*) was thought to be the causal organism. Orton and Adams (8) also reported on a collar blight of various varieties of apple trees in Pennsylvania which they considered was caused by the fire-blight organism. In certain regions where Grimes Golden and other varieties susceptible to trunk canker were grown, most collar disorders were formerly attributed to fire blight. As a result of such diagnoses, some effort was expended in attempting to eradicate the twig form of the fire blight before it spread to the collar.

The work of Baines (1) demonstrated that this type of collar blight and trunk canker on the Grimes Golden variety was caused not by the fire-blight organism but by *Phytophthora cactorum* (Leb. and Cohn) Schroet. Welsh (13) has recently studied collar injury of apple trees in British Columbia and attributed it to *Phytophthora cactorum*, possibly a strain of the organism causing collar blight.

No other intensive studies have been reported on collar and root disorders of apple in the region south of New York State. In this more southern region winter injury to the above-ground parts is usually slight, but at the collar it may be extensive.

The researches of Carrick (2), Chandler (3), Potter (9), and Magness (7) show that root tissue of seedling apple trees when exposed in a cold

[1] Respectively, senior pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture; and Plant Pathologist, Virginia Agricultural Experiment Station.

chamber is more susceptible to cold injury than is the stem tissue. The work of these authors indicated that injury to roots occurs at from 19° to 17° F.

The studies on which this paper is based were made in the central and southern Appalachian region (North Carolina, Virginia, West Virginia, and Maryland) and also in Indiana, New York, and Pennsylvania. The trees showing winter injury of the collar and root were therefore growing under a great variety of soil environments as well as of climatic conditions.

Winter injury, for the present purpose, may be regarded as the effect of freezing temperature on trees that are in a susceptible condition. Susceptibility of apple is assumed to be caused by one or more of a number of contributing factors prior to and during the season in which the injury appeared. The principal factors concerned are drought, over-bearing, poor root aeration because of a waterlogged soil, immaturity in the fall, premature defoliation caused by leaf diseases or by early frosts in the fall, and breaking of winter dormancy followed by severe cold.

DESCRIPTION OF ROOT AND COLLAR INJURY

The injury varies from a shallow lesion (Fig. 2, A, B, D), in which only the cortex is affected, to a deep lesion (Fig. 1, A) extending through the cambium. Both types of injury are usually located at the collar, but adjacent roots may also be involved. The injury usually begins ½ to 1 in.

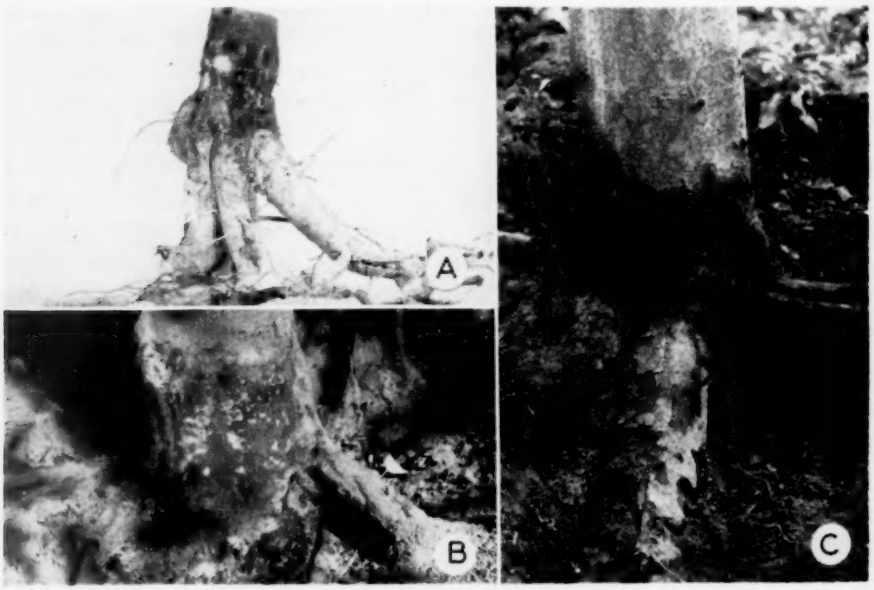

FIG. 1. A. Constriction and depressions about the collar region of an apple tree resulting from the healing of winter-killed areas which in some spots extended through the cambium. B. Islands of living tissue within a winter-injured area at the junction between the dead stock and the still living scion. C. Root system of a young bearing tree completely killed by the combined influence of low temperatures and wet soil.

below the soil line. The point where the injury occurs is close enough to the surface of the ground so that there is little protection from the soil covering.

FIG. 2. A, B. Superficial killing and healing of collar tissues of mature apple trees. Margins of the lesions have been rendered conspicuous by scraping the area until the highest points of living tissue have been revealed. The cambium was not destroyed. C. Rootstock of a young tree completely killed, apparently because of lack of hardiness of the stock. D. Shallow, longitudinal collar injury on a young apple tree. The dead tissue has been removed to show the extent of the lesion. The cambium was uninjured.

In the case of the shallow lesion only the outer portion of the bark is killed. The lesion may be no more than an inch in diameter, or it may extend laterally for several inches. These shallow lesions usually heal over completely with little or no apparent injury to the tree. Since they can be seen only after some of the soil has been removed, they are seldom detected. The shallow type of lesion occurs more frequently than the deep lesion, and often it is the only type observed.

The deep lesion involves phloem and cambium and may vary from 2 in. in diameter to a size sufficient to girdle the tree. When a considerable portion of the phloem is destroyed, that part of the root which is beyond the lesion must of necessity soon die. The killing often is not uniformly deep. In such lesions there are usually spots where the cambium is killed, and also areas of varying size where it is not killed. Examination subsequent to a period when growth could take place reveals islands of live bark within the injured area. These islands are usually present during the summer immediately following winter injury. Sometimes in severe winter injury the killing is uniform throughout and there are no islands of living bark tissue. These severe cases are usually associated with abnormally low temperatures or with a waterlogged soil in conjunction with low temperatures.

The islands of live tissue in the dead bark of a lesion are sufficiently prevalent and characteristic of collar winter injury to be considered an important diagnostic characteristic. Steinmetz and Hilborn (10) found that well hardened-off cambium tissue of apple trees is more resistant to cold injury than the cortex.

Another distinguishing feature characteristic of winter injury, especially in the early stages, is wet and loose, sloughing bark in which the cambium seems to be disintegrating. This is in contrast to the tight, clinging bark of a lesion caused by a root pathogen. In the latter case there is usually some evidence of the fungus mycelium on the surface of the lesion or within the bark.

Both the deep and the shallow types of winter injury are usually confined to one side of the tree; and in both types the lateral extension is usually greater than the vertical. The lesions may extend only 3 inches up and down, but far enough laterally to involve as much as a quarter or more of the circumference of the trunk.

DISTINCTION FROM PATHOGENIC ROOT ROTS

In view of Welsh's report (13), it seemed desirable to examine collar rots of apple trees over a wide range of environment in the eastern United States in order to determine to what extent *Phytophthora* is the causative agent. Repeated but unsuccessful attempts to isolate *Phytophthora* from apple collar lesions were made by the writers and also by associates experienced in culturing this fungus. The method used most frequently was that reported by Tucker (12), *i.e.*, inserting into an apple fruit a piece of bark taken from the margin of the dead tissue. Drechsler's method (5) of plac-

ing the test material in water, so that the fungus may outgrow contaminating bacteria, was also used. Moreover, careful microscopical examination of sections through the margin of diseased tissue showed no fungus mycelium.

Further evidence that this trouble is not caused by a pathogen is the location of the lesions on one particular side of the tree, as is often the case with other types of winter injury. Lesions resulting from a pathogen are more often indiscriminately located. The irregularity in depth of the lesions also indicates that they are not caused by a pathogen. In the case of the deep lesions, the islands of live tissue that grow during the first summer after the injury occurs furnish the most conclusive evidence that the lesions are not caused by a pathogen. Islands of live tissue have never been noted in the lesions on apple produced by the pathogens *Xylaria mali* Fromme, *Corticium galactinum* (Fr.) Burt., *Sclerotium rolfsii* Sacc., *Rhizoctonia* sp., or *Phytophthora cactorum* (causing trunk canker), nor in those caused by *Armillaria mellea* Vahl ex Fr. on stone fruits. The nature of the healing process gives further evidence of the nonpathogenic nature of the lesion. When no pathogen is present a healing callus grows around the margin of the wound, thus making the lesion smaller. In contrast to this, a lesion caused by a pathogen usually continues to enlarge.

PREDISPOSING FACTORS

The observations of the writers indicate that collar winter injury is influenced by certain environmental and nutritional conditions, briefly reported here. In this respect it is probably similar to other types of winter injury.

Nutritional conditions and maturity. A tree weakened by inadequate feeding may be more susceptible to collar winter injury than a well-fed tree. The production of an excessive crop of fruit has a similar effect in depleting nutrients of the tree and increasing susceptibility to various types of winter injury. Closely allied with nutrition is the question of tissue maturity and "hardening" at the onset of winter weather. Any weather or nutritional conditions unfavorable for proper tissue maturity and winter hardiness are conducive to collar winter injury.

Poor aeration of the roots. Field observations indicate that collar winter injury may be prevalent when there is excessive moisture during the winter, especially when a wet period is followed by a severe cold period. In such cases it is difficult to say how much of the injury was caused by poor aeration and how much by the low temperature; at any rate, the two adverse conditions working together give much more severe injury than either working alone. Orchards located on loose mountain soil may have little or no collar injury, whereas trees located near by in clay soil may have much of it.

Sometimes this type of injury is definitely related to topography. One orchard on fairly level land, where losses had occurred from root troubles,

had slight depressions in the surface, and tree replacements were largely confined to these low spots. Trees remaining on the edge of a low spot were affected later and less severely than those in the center of the depression. The evidence indicates that during a wet spell there was slight seepage and poor drainage in this low spot. Here the cold injury was severe enough to girdle the trees at the collar, while trees situated more favorably recovered from any slight injury that occurred. Definite symptoms of collar winter injury can often be noted on trees growing in a swale, whereas such injury is very slight or absent in the higher part of the same orchard.

Root killing that was apparently in large measure caused by poor aeration of the soil following excessively heavy rainfall developed in an apple orchard in West Virginia in 1943 (Fig. 1, C). Approximately 800 six-year-old trees were either dead or dying by June. A number of the trees were removed and the root systems examined. The major amount of tree killing occurred in areas where the trees had been heavily mulched with strawy manure. The over-vigorous condition of the trees, the immaturity of wood associated with late-season growth, and a near-saturated soil seemingly were the principal contributing factors. Bark destruction was complete from the ground line to a depth of approximately 1 ft. on the most severely affected trees. Other trees had only a few roots killed, yet it seemed typical that any root affected was entirely killed. In less severely affected trees, the injury became evident as retarded growth made the trees conspicuous. Many such weakened trees have since died or been removed.

Lack of winter-hardiness of rootstock. Another type of what is apparently collar winter injury in young trees seems attributable to lack of hardiness of the rootstock. The rootstock dies, without symptoms indicative of pathogenic invasion, drowning, or other discernible cause. The killing extends from the stock-scion union downward and involves all portions of the root system (Fig. 2, C). Trees from 2 to 10 years of age succumb suddenly without prior evidence of distress. It seems significant that this type of rootstock killing has not been observed in mature trees. Susceptible stocks apparently succumb within a few years after planting. Affected trees have been observed in various topographic situations and no correlation was apparent with the usual predisposing factors, such as wet soil, location with respect to natural cold air stratification, cultural or fertilizing practices, *etc.* Replants commonly survive in the same location without development of similar injury. Adjacent trees in young plantings usually continue to develop without signs of similar injury. While it is admitted that the evidence for lack of hardiness on the part of the stock is wholly circumstantial, the striking variation in character of seedling stock seems an adequate explanation under the circumstances.

Incompatibility of stock and scion must always be considered when young trees die without any apparent cause. The symptoms of incompatibility are usually sufficiently distinct that one can distinguish it from collar winter injury. In case of incompatibility of stock and scion the outward

distress symptoms, usually appearing in the tree tops and particularly at the tips of the branches, are associated with a gradual decline of the fibrous roots and with a failure to produce new roots. In case of collar or root winter injury, however, the collar and larger roots are the first to become necrotic.

Warm weather in winter. A period of warm weather in midwinter, such as often occurs in the southern range of apple production, may start growth processes to such an extent that subsequent cold weather, perhaps even a slight freeze, will produce collar winter injury. Such a sequence may be responsible for the extensive collar winter injury in the mountains of southern North Carolina and northern Georgia.

DISCUSSION

Collar winter injury is often overlooked by the casual observer because it occurs underground. Probably many more apple trees have the symptoms than most orchardists or even pathologists realize.

Most of the severe winter injury has been reported in the northern States. In milder climates, as in Georgia, North Carolina, Tennessee, and Virginia, healing often follows mild winter injury and allows the tree to continue to function; even in cases of severe injury the tree usually does not die until midsummer and the orchardist may not suspect winter injury as the cause of death.

The work reported in this paper indicates that collar winter injury of apple trees is the resultant of a number of concomitant climatic and physical factors, with a consequent physiologic condition of susceptibility to cold injury. For technical use, "physiocheimic injury," derived from physio (physiological) + cheimic (pertaining to winter), is suggested to replace the more general term "winter injury" as a designation for the type of cold injury in which the physiological condition of the affected plant is an important determining factor.

SUMMARY

Injuries to the collar and the root system of apple trees in the central and southern parts of the apple-growing region of the eastern United States have been described. Evidence is presented which indicates that these injuries are not the result of primary action of a pathogen, but are caused by complex effects of low temperature and certain other environmental circumstances which have increased susceptibility to cold. The term "physiocheimic injury" is suggested to denote this condition.

PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

1. BAINES, R. C. Phytophthora trunk canker or collar rot of apple trees. Jour. Agr. Res. [U. S.] 59: 159–184. 1939.
2. CARRICK, D. B. Resistance of the roots of some fruit species to low temperature. N. Y. (Cornell) Agr. Exp. Sta. Mem. 36: 609–661. 1920.

3. CHANDLER, W. H. The killing of plant tissue by low temperature. Mo. Agr. Exp. Sta. Res. Bul. 8: 141–309. 1913.
4. COOLEY, J. S. Root diseases of deciduous fruit trees. Bot. Rev. 12: 83–100. 1946.
5. DRECHSLER, CHARLES. The beet water mold and several related root parasites. Jour. Agr. Res. [U.S.] 38: 309–361. 1929.
6. GROSSENBACHER, J. G. Crown rot of fruit trees. Field studies. N. Y. (Geneva) Agr. Exp. Sta. Res. Bul. 23: 1–39. 1912.
7. MAGNESS, J. R. Collar rot of apple trees. Wash. Agr. Exp. Sta. Bul. 236. 1929.
8. ORTON, C. R., and J. F. ADAMS. Collar blight and related forms of fire blight. Pa. Exp. Sta. Bul. 136. 1915.
9. POTTER, G. F. Experiments on resistance of apple roots to low temperatures. N. H. Agr. Exp. Sta. Tech. Bul. 27. 1924.
10. STEINMETZ, F. H., and M. T. HILBORN. A historical evaluation of low temperature injury to apple trees. Maine Agr. Exp. Sta. Bul. 388. 1937.
11. THOMAS, H. E. Root and crown injury of apple trees. N. Y. (Cornell) Agr. Exp. Sta. Bul. 448. 1926.
12. TUCKER, C. M. Taxonomy of the genus Phytophthora de Bary. Mo. Agr. Exp. Sta. Res. Bul. 153. 1931.
13. WELSH, MAURICE F. Studies of crown rot of apple trees. Canad. Jour. Res. (C) 20: 457–490. 1942.

STUDIES ON SAFFLOWER RUST IN INDIA[1]

RAGHUBIR PRASADA AND HOMI P. CHOTHIA

(Accepted for publication October 20, 1949)

Safflower, *Carthamus tinctorius* L., is one of the important oil-seed crops in India. The plant is used as fodder and the seed for extracting an oil which is of great economic value.

Three rusts have been reported on this crop, *viz.*, *Puccinia carthami* (Hutz.) Corda, *P. verruca* Thüm., and *Aecidium carthami* Dietr. In India only *P. carthami* has been found on the cultivated species *Carthamus tinctorius* L. and the wild species *C. oxyacantha* Bieb.

Although *Puccinia carthami* has been reported in India by Butler and Bisby (2), no systematic study has been made so far, with the result that there is hardly any information on the factors which cause the recurrence of this disease. Normally, safflower is sown in October or November and harvested in April. The rust on the cultivated crop appears in February or March as pycnia which soon are followed by uredia. Teliospores form in the same sori as the urediospores, at the end of the season when the host plants are mature.

On the wild host, *Carthamus oxyacantha*, telia are observed as late as July and August or until the plants perish. In the first week of January pycnia and uredia are observed on the cotyledons of some of the seedlings that come up in December. At that time there is no infection on the cultivated crop. Thus, the rust appears nearly a month earlier on the wild host than on the cultivated one. The uredia continue to form until the middle of May.

Cotyledons, leaves, and sometimes tender stems are infected. Sori are amphigenous, scattered, erumpent, and chestnut brown. The urediospores are globoid or broadly ellipsoid; their wall is light chestnut brown, 1.5μ to 2μ thick, and finely echinulate. The urediospores from the wild as well as the cultivated safflower measure 21–28 × 19–25μ and have three to four equatorial germ pores.

Fresh urediospores start germinating in 4 hr. at 18° C. in a moist chamber. Over a temperature range from 8° to 35° C., 60–70 per cent of the spores germinate at 8°–10° C., 70–80 per cent at 12° C., 80–90 per cent at 18°–20° C., 5 per cent at 29°–30° C., and only a trace germinate at 35° C. No germination has been observed at 40° C. Stored dry at 8°–10° C., the urediospores remain viable for over a year, whereas they did not germinate after 3 weeks in April at room temperature, where the maximum was 35° C. When infected plants are exposed to high temperatures, the urediospores remain viable for 3 weeks at 30°–31° C., 20 days at 36°–37° C., 13 days at 40°–41° C., 4 days at 45°–47° C., and only 3 days at 52°–55° C.

[1] The authors are grateful to Dr. R. S. Vasudeva, Head of the Division, for his valuable suggestions.

Inoculations made during September and October produce infection in 12–14 days; and those made between November and March in 10–13 days. At 18°–20° C. the incubation period is 10 days which appears to be the shortest. At high temperature (40° C.) the rust shows a tendency to form telia directly without producing any urediospores in the sorus. Temperatures in May at Delhi are unfavorable for the germination of urediospores and hence the culture cannot be maintained.

Cross inoculations with urediospores from *Carthamus tinctorius* and *C. oxyacantha* produce heavy infection. This fact and morphological similarity go to show that the rust found on the two is one and the same.

The uredial stage is followed by the telial, the spores being formed in the same sorus and on the same mycelium. In nature, the teliospores are generally formed when the host reaches maturity and environmental conditions are unfavorable for the propagation of the rust in the uredial stage. The teliospores are the resting spores in rusts but they may or may not require a dormant period, depending on the rust and the environmental conditions. Beyond what Prasada (6) reported, there is no information regarding a resting period for teliospores of *Puccinia carthami*, their viability under different environments, and the precise temperature that permits their germination. Since this information is necessary to determine the role of teliospores in the annual rust outbreaks, infected leaves of *Carthamus tinctorius* were collected from the field in April and were stored in sealed glass tubes at 3°–5° C., 8°–10° C., 14° C., and 17°–18° C. A part of this collection was exposed to natural conditions and the rest kept in the laboratory at Delhi.

Soon after collection in April, infected leaves of *Carthamus tinctorius* were soaked in water overnight and teliospores kept for germination at 7°–8° C., 12°–14° C., 17°–18° C., 20°–22° C., 26° C., 29° C., and 30°–35° C. As observed by Prasada (6), the best germination was between 12° and 18° C. and a few spores germinated at 20°–22° C.; but no germination was observed at 7°–8° C. and at 26° C. and above. All germination tests were, therefore, made at 12°–18° C. It has been found that a small percentage of spores germinate in April and May soon after formation. During the following 5 months no germination is observed, but in November the spores germinate profusely. These tests were always made at 12°–18° C. with the same result. It has also been observed that some of the teliospores appear to be empty in November, as if they had germinated at an earlier date. From November to January, approximately 60–80 per cent of the teliospores germinate, but from February to March there is a gradual decline in germination. Teliospores formed in culture during winter germinate better and more consistently than those formed in nature in April. Exposure to summer heat in Delhi (which reached 40° to 45° C. during May and June, 1948) does not kill all the teliospores, but storage at lower temperature is more conducive to the retention of viability.

When teliospores from *Carthamus oxyacantha*, the wild safflower, were tested for germination soon after collection in July and August, 5–10 per cent germinated, after which no germination was observed till December. These spores germinated profusely in January. As in the case of teliospores from *Carthamus tinctorius*, temperature between 12° and 22° C., with optimum at 12°–18° C., has been most suitable for germination.

From these observations, it appears that two types of teliospores are formed in this rust: one type with a limited period of viability and with ability to germinate soon after formation, and the other type that requires a resting or maturation period. Morphologically they are alike except for the lighter color of the former.

On germination both cells of the teliospore produce a stout four-celled promycelium, each cell of which gives rise to a basidiospore on a short sterigma. The basidiospores are hyaline and kidney-shaped. On germination they give rise to a thin and short germ tube.

The technique employed for inoculation with teliospores was essentially the same as described by Newton and Johnson (5). Inoculations made during September did not succeed. Results are positive, however, in November when pycnia appear after 10 days and uredia are formed 3 days later. The fungus is an autoecious, brachyform rust. Similar results are obtained in December, January, and February, but inoculations made towards the end of March, when the temperature is above 26° C., give negative results. Cross inoculations with germinating teliospores on *Carthamus tinctorius* and *C. oxyacantha* produce infection.

In order to determine whether the infected bits of plants lying in the field from the previous crop would cause infection, seed of *Carthamus tinctorius* was sown in November in sixteen pots which were divided into four groups of four pots each. Rusted leaves which had been exposed to natural conditions through the summer were mixed with the surface layer of soil in the pots of the first group; the second group received rusted leaves that had been kept in the laboratory room; to the third group were added rusted leaves that had been stored at 8°–10° C.; and the fourth group was left untreated as controls. All the pots were covered with muslin bags to safeguard against wind-borne infection. Pycnia and uredia appeared on lower leaves and stems of seedlings in the first three groups, when they were 3 to 4 weeks old, whereas seedlings in the controls remained healthy. Thus, plant debris from the previous crop can furnish inoculum for fresh infections of a new crop.

The following experiments were also conducted to determine whether the rust could be perpetuated by means of teliospores carried on the seeds. Seeds of *Carthamus tinctorius* were first surface-disinfected with 0.1 per cent mercuric chloride solution for 5 min. and then washed in distilled water. Some of these seeds were mixed with teliospores and sown in pots. The rest were sown in separate pots as a control. All the pots were covered

with muslin bags. After 3 to 4 weeks, pycnia and primary uredia developed on the plants that were raised from infected seed, whereas plants in the control remained healthy, showing that teliospores carried with the seed can start new infections in the following year. Conners and Savile (4) have made similar observations in Canada.

Agrosan GN, a volatile organo-mercury compound, has been found by us to be toxic to the teliospores adhering to the seed. Seed treatment with this fungicide at the rate of 3:1,000 completely controlled externally seed-borne infection of the seedlings.

DISCUSSION

Puccinia carthami attacks the cultivated safflower, *Carthamus tinctorius*, and the wild species, *C. oxyacantha*. The cultivated safflower is sown in October or November and harvested in April or May, but the wild species is found until August, after which its aerial parts disintegrate. Morphological as well as pathological tests show that the rust on both species is identical and capable of infecting either host. Since the rust has been observed earlier on the wild species than on the crop, it appears that the cultivated crop is infected by inoculum from the wild plants. The reaction of urediospores to temperatures above 40° C. and the fact that cultures of this rust could not be maintained after April, and also, the tendency of this rust to produce telia directly at high temperatures without any urediospores in the sorus, show that oversummering of this rust in the uredial stage is most unlikely.

It has been shown that two types of teliospores are formed in this rust: one type with a limited period of viability and with ability to germinate soon after formation, and the other type that requires a resting or maturation period. According to Arthur (1), in some species of rusts all the teliospores are developed as resting spores, but in others, *e.g., Puccinia triticina*, only a part of them are so constituted. In the majority of species no conspicuous structural difference is evident to distinguish such spores. Chester (3) has made a similar observation. When he examined the oversummered teliospores of *P. triticina* he found that a considerable number of cells had germinated at some time between their formation and the date of their collection. This was indicated by empty cells with well-marked holes in the cell walls through which the promycelium had emerged. We have also made similar observations in the rust under study. These evidently were the teliospores which, according to Arthur (1), do not require a resting period.

It is the resting teliospores that oversummer and remain viable to bring about fresh infection in the following season. Inoculum may come from plant debris lying in the soil or it may be carried with the seed in the form of tiny bits of infected tissue. Teliospores from wild safflower may infect the cultivated species directly or may attack the wild species first since the rust has been observed earlier on the wild plants than on the cultivated ones.

It should, therefore, be possible to control this rust to a considerable extent by burning the plant debris after harvest and treating the seed with Agrosan GN, and by the eradication of the wild *Carthamus oxyacantha.*

SUMMARY

The rust *Puccinia carthami* was observed on *Carthamus tinctorius,* the cultivated safflower, in February and March and on *C. oxyacantha,* the wild species, in January near Delhi, India. It was found to be identical, morphologically as well as pathologically, on both hosts, and cross inoculations were successful.

The urediospores germinate well between 8° and 30° C. with optimum at 18°–20° C. Stored dry at 8°–10° C., they remain viable for over a year, whereas those kept in the laboratory in April lose their viability after 3 weeks. When infected plants are exposed to high temperatures, the urediospores remain viable for 3 weeks at 30°–31° C. and for only 3 days at 52°–55°C.

The incubation period is 10–14 days depending on temperature. At temperatures above 40° C. the rust shows a tendency to form telia directly without the formation of urediospores in the sorus. Rust cultures cannot be maintained in May in Delhi and the urediospores are unable to oversummer.

The teliospores are formed in April. Some of them germinate soon after formation, while others require a resting period of 5 or 6 months. All of them appear to be alike and germinate well between 12° and 18° C. Cross inoculations with teliospores produce pycnia and uredia in 10–14 days. Resting teliospores oversummer and remain viable to bring about fresh infection in the following year. Teliospores from wild safflower may infect the cultivated species directly or may attack the wild species first.

INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI, INDIA

LITERATURE CITED

1. ARTHUR, J. C. The plant rusts (Uredinales). 446 pp. John Wiley & Sons, New York. 1929.
2. BUTLER, E. J., and G. R. BISBY. The Fungi of India. Sci. Monogr. Coun. Agr. Res. India 1: 65. 1931.
3. CHESTER, K. STARR. The Cereal Rusts. 269 pp. Chronica Botanica Company, Waltham, Mass. 1946.
4. CONNERS, I. L., and D. B. O. SAVILE. 23rd Ann. Rept., Canad. Plant Disease Survey, 1943. xviii + 122 pp. 1944. (Mimeographed.)
5. NEWTON, M., and T. JOHNSON. Specialization and hybridization of wheat stem rust. Bul. Canada Dept. Agr. 160. 1932.
6. PRASADA, R. The rust of safflower, *Puccinia carthami* (Hutz.) Corda. Curr. Sci. **16**: 292. 1947.

VERTICAL DISTRIBUTION OF VERTICILLIUM ALBO-ATRUM IN SOILS

STEPHEN WILHELM

(Accepted for publication November 3, 1949)

Verticillium albo-atrum Rke. et Bert. is one of the most important soil-borne vascular parasites of higher plants. Its extensive host range, which includes important agricultural crops, and its wide distribution throughout the temperate countries of the world, emphasize its importance. Control measures, apart from utilization of genetic resistance, must be directed at the soil, and to this end considerable effort is being expended.

Information about the vertical distribution of pathogenic soil fungi is essential before the most effective use can be made of soil fumigants. The selection of soil fumigant, dosage, and method of application, including possible measures taken to seal the soil against surface loss, must be governed in part by the vertical distribution of the pathogen in question.

It has been shown by many workers (6, 11, 12, 14, 15) that although fungi predominate in the upper 8 to 12 in. of the soil, they are by no means limited to these depths. Species of *Trichoderma* and *Zygorrhynchus* commonly inhabit the subsoils. That Fusaria may occur in certain nursery soils to a depth of 24 in. was demonstrated in 1917 by Taylor (13). Leach and Davey (5) have shown that *Sclerotium rolfsii* Sacc. predominates in the upper 6 in. of the soil, and only rarely occurs deeper than 1 ft. On the other hand, in cultivated land *Phymatotrichum omnivorum* rarely occurs at a depth less than 6 in. King and Hope (4) have shown that the greatest density of Phymatotrichum sclerotia occurs below the 12-in. depth, the peak of concentration being between 16 and 20 in. For the eradication of small outbreaks of Verticillium wilt in hop plantations Keyworth (3) has recommended removal of the soil of the infested area to a depth of 3 ft. Keyworth apparently assumed that *Verticillium albo-atrum* might occur as deep as 3 ft. *Armillaria mellea* (Vahl.) Quel. and *Rosellinia* (*Dematophora*) *necatrix* Hart. have both frequently been isolated from the roots of dead orchard trees at a depth of 6 to 7 and occasionally 11 ft.[1]

INFECTION OF A SUSCEPTIBLE HOST USED TO MEASURE THE INFESTATION

Inasmuch as the microsclerotia of *Verticillium albo-atrum* are too small to be screened or washed from soil, use could not be made of the techniques applied in the vertical distribution studies of *Sclerotium rolfsii* or *Phymatotrichum omnivorum*. Infection of a susceptible host such as the Bonny Best variety of tomato, however, was found to give a reliable indication of an infestation, and also lent itself to a quantitative interpretation.

Soil samples were obtained from fields known to be infested with *Verti-*

[1] From unpublished data of Dr. Harold E. Thomas.

cillium. Each sample was obtained by digging a large hole to 3 ft. and removing from each 6-in. depth interval enough soil to fill a 5-gal. can. Care was taken to prevent contamination of the deeper soil layers with surface soil. All cans used were autoclaved. The shovel used for digging was washed periodically in a 4 per cent solution of formaldehyde. When the field being sampled was in furrow cultivation at the time the sample was obtained, measurement was made from the top of the ridge.

At the greenhouse, four 8-in. sterilized clay pots were filled from each 5-gal. sample of soil, and 10 Bonny Best tomato seedlings were transplanted into each pot. Thus a total of 40 plants was grown in the soil of each 6-in. depth interval of a sample. The presence of *Verticillium* was indicated by the infection of the plants. A consistent planting pattern was followed in all but the first four samples. Eight seedlings transplanted in the cotyledon stage were equally spaced around the edge of the pots and two were spaced in the center. The plants were fed when necessary with a mixture of equal parts of a nitrogenous commercial fertilizer and ammonium sulfate. At the end of a growing period of from 6 to 8 weeks (6 in summer, 8 in winter), each plant was cultured at the crown for *Verticillium*. In culturing, a plain agar medium containing bits of dried plant materials as nutrients, described by Snyder and Hansen (9), was used. The portion of the stem to be cultured was first surface-sterilized by immersion in a 1:1000 solution of mercuric chloride in 10 per cent ethyl alcohol. Complete cross sections of the stems were used in culturing to insure a representation of all of the vascular elements of the stem. It should be stated here that under the conditions of this experiment the absence of symptoms did not coincide in all cases with the absence of infection, and consequently it was necessary to culture each plant.

The presence of *Verticillium* and the relative quantity found at the various soil depths are indicated by the infection index of the soil sample. The infection index is defined as the percentage of the plants, planted in a unit volume of soil (in this instance, soil of a 6-in. depth interval filling four 8-in. pots), from which *Verticillium* is isolated after a growing period of 6 to 8 weeks. The infection index can be determined for a single 8-in. pot of soil or for several, depending upon the volume of soil being so analyzed.

RESULTS

The results, presented in table 1 and figure 1, are based on depth samples from 20 sites in coastal and interior districts of California from land of a variety of soil types and with a variety of crop histories. The soils varied from heavy clay loam to loam in the coastal counties, and from sandy loam to nearly pure sand in the interior valley counties. The subsoils in the coastal counties varied from adobe to nearly pure sand, and in the interior valley counties from sandy loam to heavy clay. In at least half of the samples the surface soil to a depth of 6 in. or more was dry and dusty at the

time the sample was taken. Samples 4 and 18 were obtained from areas that had been filled with surface soil.

The crop histories, as far as could be determined, were as follows: samples 2 and 19, several years of tomato; 10, two years of dry fallow following tomato; 20, tomato following alfalfa; 1, 3, and 4, boysenberry; 9, raspberry; 7, 8, 17, and 18, strawberry following tomato, with eight years of grain between in 17 and 18; 5, potato; 6, potato following tomato; 11 and 12, boysenberry following cotton; 13–16, cotton. Samples were collected in the counties of California as follows: 1–4, Santa Cruz; 5–6, San Benito; 7–10, Santa Clara; 11–12, Tulare; 13–16, Kern; 17–18, Alameda; 19–20, San Joaquin.

In table 1 the complete data for five of the depth samples selected at

TABLE 1.—*Presence of Verticillium in soil samples from different depths as determined by infection of tomato*

Depth in inches	Number of plants out of 10 per pot, infected with Verticillium. A, B, C, D represent 4 plots containing soil of one 6-in. depth interval of a sample					
	Sample and infection index	Sample number				
		2	6	12	17	18
0–6	A	10	3	10	0	9
	B	9	5	10	1	6
	C	9	5	7	0	9
	D	10	4	10	1	6
	Index	95.0	42.5	92.5	5.0	75.0
6–12	A	8	8	1	2	7
	B	9	7	1	0	8
	C	6	6	1	1	6
	D	5	7	3	1	6
	Index	70.0	70.0	15.0	7.5	62.5
12–18	A	1	1	2	0	0
	B	1	0	1	0	1
	C	1	0	1	0	1
	D	0	2	1	0	1
	Index	7.5	7.5	12.5	0.0	7.5
18–24	A	1	0	2	0	0
	B	0	0	1	0	0
	C	0	0	2	0	0
	D	0	0	1	0	0
	Index	2.5	0.0	15.0	0.0	0.0
24–30	A	1	0	0	0	0
	B	0	0	0	0	0
	C	0	0	0	0	0
	D	1	0	1	0	0
	Index	5.0	0.0	2.5	0.0	0.0
30–36	A	0	0	0	0	0
	B	0	0	0	0	0
	C	0	0	0	0	0
	D	0	0	0	0	0
	Index	0.0	0.0	0.0	0.0	0.0

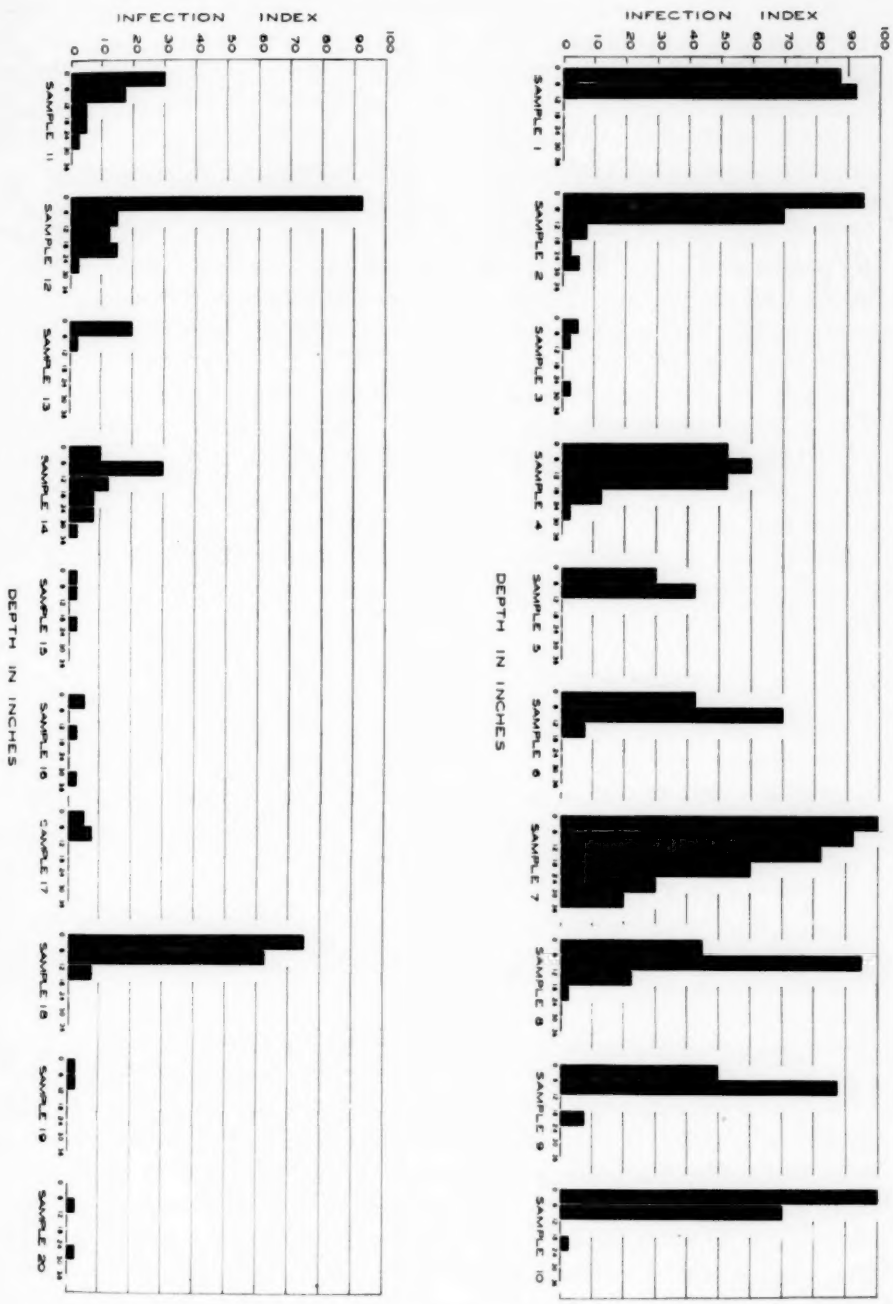

FIG. 1. Infection indices of soil depth samples indicating the quantitative vertical distribution of *Verticillium albo-atrum*. Each bar represents a 6-in. depth interval beginning at 0–6 in. (left). Samples are from the respective counties of California as follows: 1–4, Santa Cruz; 5–6, San Benito; 7–10, Santa Clara; 11–12, Tulare; 13–16, Kern; 17–18, Alameda; 19–20, San Joaquin.

random are shown. These data are representative of the kind obtained for the 20 samples and illustrate the fairly uniform distribution of Verticillium inoculum throughout the four pots of soil of a given depth interval. They also illustrate the great differences in numbers of plants infected which occurred, particularly between the 6–12- and 12–18-in. depths. Figure 1 gives the infection index for each 6-in. depth of the 20 samples, each bar on the graph representing a 6-in. depth interval.

In certain of the first samples studied, *viz.*, numbers 1, 8, 9, 10, before the method was completely standardized, a similar number of plants was not always planted in each pot, and where two or more plants of a pot failed to grow, the infection index was corrected on the basis of a total of 40 indicator plants.

In samples of 20 soils, *Verticillium* was detected in the 0–6-in. depth in 19, in the 6–12-in. depth in 19, in the 12–18-in. depth in 10, in the 18–24-in. depth in 10, in the 24–30-in. depth in 8, and in the 30–36-in. depth in 3. The 0–6 and 6–12-in. layers were found on the whole to contain 3 to 4 times the degree of infestation of the deeper soil layers. There appears to be no relation between the type of soil, surface or subsoil or crop history of the land including depth of root penetration, and the vertical distribution of *Verticillium albo-atrum.*

RELATION BETWEEN INFECTION INDEX AND INOCULUM POTENTIAL OF THE SOIL

Early in the progress of this study it became apparent that the infection index should be related to the amount of inoculum per unit of soil, that is, to the inoculum potential of the soil. Theoretically, in the case of the ideal soil invader, exemplified perhaps by an obligate parasite such as *Plasmodiophora brassicae* Woron., as Samuel and Garrett (8) have shown, a determination of the number of independent infections can be used to give a measure of the amount of the fungus in the soil, or at least of the activity of the fungus at a particular time. It was conceived, in the case of *Verticillium albo-atrum,* that the percentage of test plants infected in a given soil within certain time limits would give an approximation of the amount of the fungus present in the soil. This assumption was tested in the following experiments.

The general plan of the experiments was to inoculate a standard volume of noninfested soil with different amounts of Verticillium inoculum, and to determine within given time limits whether differences in amount of inoculum (inoculum potential) were reflected in the infection index. The standard volume of soil used in these experiments was that contained by an 8-in. pot, and the standard unit of inoculum, a piece of tomato stem tissue 10–15 × 2–3 mm. upon which *V. albo-atrum* had been grown for 2 weeks. In the preparation of the inoculum the tomato stem pieces were sterilized by fumigation with propylene oxide (2), and then inoculated by dipping them into a spore suspension. Within 3 to 5 days after inoculation, the tomato

stem pieces became covered with microsclerotia. The test plants in these experiments were seedlings of the Bonny Best variety of tomato transplanted in the cotyledon stage, 10 per pot in the pattern mentioned. The growing time was 7 to 8 weeks.

A series of 5 pots was inoculated with 1, 2, 10, and 25 Verticillium inoculum units each in three different trials. In the first trial the soil used had been fumigated with chloropicrin (0.5 cc. per gal.) and aired 2 weeks; in the second, tomatoes were first grown for 6 weeks in chloropicrin-fumigated soil; and in the third trial a virgin mountain soil was used. The results are shown in table 2. The infection index is calculated on the basis of 50 indicator plants in five 8-in. pots of soil.

TABLE 2.—*Test to determine the relation of infection index to inoculum potential*

Trial	Number of inoculum units per pot			
	1	2	10	25
	Infection index			
I	0.0	0.0	10.0	12.0
II	0.0	0.0	12.0	52.0
III	2.0	10.0	24.0	66.0

In trial 1, with previously fumigated soil, it was suspected that some factors, probably biological, were responsible for inactivating the inoculum, because such low infection indices were obtained. This suspicion is borne out by the higher indices obtained in trial 2 with the same soil after it was cropped to tomatoes, but it was still not possible to detect the inoculum of pots inoculated with only 1 and 2 units. Trial 3, with untreated soil, gave the most significant results, the mean infection indices, 2.0, 10.0, 24.0, and 66.0 respectively, for soil inoculated with 1, 2, 10, and 25 units, showing a direct relationship between infection index and inoculum potential.

All of the pots with initial rating of 0, except those of trial 2, were planted a second time. In 7 of the 21 pots rated 0, the second planting rather than the first indicated the presence of the infestation. In no instance, however, were more than two plants of the 10 per pot infected.

In another test, the total amount of inoculum per pot was kept constant, but the unit size and distribution of the inoculum were varied. Sets of five 8-in. pots of soil were each infested with the following amounts of inoculum: a single large piece of tomato stem inoculum and the same-sized piece cut into 2, 10, and 25 pieces respectively. Where 25 pieces were used, each was approximately equal to one standard inoculum unit. The set of pots infested with one large inoculum unit gave an infection index of 0.0, with the same-sized piece cut into two, 6.00; into 10, 8.00; into 25, 40.0, illustrating the effect inoculum distribution can have on infection index.

DISCUSSION AND CONCLUSIONS

In assaying the inoculum potential of a soil with respect to a given pathogen in terms of infection of a suitable host, many factors must be considered. The infection index as defined in this paper must be considered a function of the inoculum potential, inoculum distribution, and past crop history of the soil. The crop history undoubtedly involves many biological equilibria, which are both difficult to assay and to maintain constant.

Another factor to be considered is the possibility that the pathogen could increase in the soil, or spread from an initial focus from root to root by contact and thus affect the infection index. It is highly questionable whether *Verticillium* can make such independent growth in soil. Nevertheless, to minimize this variable, strict growing time limits were imposed.

Many factors conceivably influence the vertical distribution of root-infecting fungi. Among the more important, depth of root penetration, antagonistic relations of other organisms, food, air, and moisture supplies may be cited. The depth of root penetration appears to be of foremost importance in influencing the vertical distribution of *Armillaria mellea* (Vahl.) Sacc. and *Rosellinia* (*Dematophora*) *necatrix* (Hart.) Berl. Vertical distribution of root-infecting fungi of the type defined by Garrett (1) as soil inhabitants (some members of the genera *Fusarium*, *Pythium*, and *Rhizoctonia*) is likely to follow that of the general fungus flora. Soil inhabitants though pathogenic are capable of making free and independent growth in the soil in competition with the purely saprophytic members of the soil microflora.

Soil invaders, exemplified by *Plasmodiophora brassicae* Woron., *Ophiobolus graminis* Sacc., *Tilletia tritici* (Bjerk.) Wint., *Mycosphaerella* (*Ascochyta*) *pinodes* (B. and Bl.) Stone and many others, usually become incorporated into a soil only with or in the plant parts invaded parasitically before death. Soil invaders are not able to compete successfully for an existence on the nonliving organic matter in the soil, nor to make independent saprophytic growth in the soil. Their survival depends upon the resisting qualities of such structures as conidia, chlamydospores, or sclerotia. Conditions which are unfavorable for the growth of the general soil microflora may favor survival of the soil invader. In arid or cold regions, for instance, resistant structures produced by soil invaders may be able to persist in the upper soil layers where the general microbial activity is greatly curtailed.

Verticillium albo-atrum cannot at present be classed definitely in either of the above arbitrary groups. In the central and southern parts of California particularly, *Verticillium* is able to persist several years (in one case studied, 8 years) in soil cropped continuously with nonsusceptible grain crops. There are indications, on the other hand, that in some regions *Verticillium* does not persist long in soils. Zeller (16) and McKay (7) reported from Oregon that a 3- to 4-year rotation of *Verticillium*-infested land with nonsusceptible crops such as grain, clover, or vetch controls Verticillium

wilt. Soloveva and Polyarkova (10) claim that rotation of cotton with alfalfa greatly reduced Verticillium wilt in the cotton.

Upon death of an infected plant, *Verticillium* promptly invades the pith and cortical tissues to produce microsclerotia in great abundance. The incorporation of such infected plants into the soil probably accounts for the high inoculum potential in the upper soil layers. The microsclerotia resist drying effectively and can withstand, for instance, a constant temperature as high as 120° F. for several months.[2] In arid regions they probably lie quiescent and viable for long periods of time.

The resistant qualities of the microsclerotia, their small size, and the fact that in cultivated lands they occur in surface soil, suggest the possibility that they could be disseminated with dust for considerable distances. Such dust dissemination might account for the infestation of lands not having a history of a susceptible crop.

SUMMARY

Quantitative information on the vertical distribution of *Verticillium albo-atrum* was obtained for a variety of soils from different regions and with different crop histories by a standardized procedure involving infection of Bonny Best tomato plants as a measure of the intensity of the infestation.

In samples of 20 soils, *Verticillium* was detected in the 0–6-in. depth in 19, in the 6–12-in. depth in 19, in the 12–18-in. depth in 10, in the 18–24-in. depth in 10, in the 24–30-in. depth in 8, and in the 30–36-in. depth in 3. The 0–6- and 6–12-in. depths were found on the whole to contain 3 to 4 times the degree of infestation of the deeper soil layers. No relation between soil type, climatic environment, or past crop history of the land to the vertical distribution of *Verticillium albo-atrum* was apparent.

Experiments were designed to relate infection index, *i.e.*, arbitrarily the percentage of Bonny Best tomato plants which become infected with *Verticillium* when planted in 8-in. pot volumes of soil, 10 plants per pot, to inoculum potential, *i.e.*, total inoculum present in a unit volume of the soil. By adding specific quantities of inoculum to untreated mountain soil and chloropicrin-fumigated soil both before and after cropping to tomatoes, it was found that within broad limits infection index is related directly to inoculum potential and is a function at least of the inoculum potential, inoculum distribution, and the past crop history of the land.

DIVISION OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

[2] Unpublished data of Mr. James Howard, University of California, Berkeley.

LITERATURE CITED

1. GARRETT, S. D. Soil as a medium for transfer and multiplication of disease organisms. Soil Sci. 61: 3–8. 1946.
2. HANSEN, H. N., and WILLIAM C. SNYDER. Gaseous sterilization of biological materials for use as culture media. Phytopath. 37: 369–371. 1947.
3. KEYWORTH, W. G. Verticillium wilt of the hop *(Humulus lupulus)*. Ann. Appl. Biol. 29: 346–357. 1942.
4. KING, C. J., and CLAUDE HOPE. Distribution of the cotton root-rot fungus in soil and in plant tissues in relation to control by disinfectants. Jour. Agr. Res. [U.S.] 45: 725–740. 1932.
5. LEACH, L. D., and A. E. DAVEY. Determining the sclerotial population of *Sclerotium rolfsii* by soil analysis and predicting losses of sugar beets on the basis of these analyses. Jour. Agr. Res. [U.S.] 56: 619–631. 1938.
6. LE CLERG, E. L., and F. B. SMITH. Fungi in some Colorado soils. Soil Sci. 25: 433–441. 1928.
7. MCKAY, M. B. Further studies of potato wilt caused by *Verticillium albo-atrum*. Jour. Agr. Res. [U.S.] 32: 437–470. 1926.
8. SAMUEL, G., and S. D. GARRETT. The infected root-hair count for estimating the activity of *Plasmodiophora brassicae* Woron. in the soil. Ann. Appl. Biol. 32: 96–101. 1945.
9. SNYDER, WILLIAM C., and H. N. HANSEN. Advantages of natural media and environments in the culture of fungi. Phytopath. 37: 420–421. 1947.
10. SOLOVEVA, MME A. I., and MME L. V. POLYARKOVA [Wilt of cotton]. Tashkent Agr. Publ. Dept. Uzbekistan Soviet Republic. 63 pp. 12 figs. 5 graphs. 1940. (Abstr. *in* Rev. Appl. Mycol. 27: 19. 1948.)
11. SWIFT, MARJORIE E. Contributions to a mycological flora of local soils. Mycologia 21: 204–221. 1929.
12. TAKAHASHI, R. On the fungus flora of the soil. Ann. Phytopath. Soc. Japan 12: 17–22. 1919. (Abstr. *in* Bot. Abstr. 5: 92. 1920.)
13. TAYLOR, MINNIE W. Preliminary note on the vertical distribution of Fusarium in soil. Phytopath. 7: 374–378. 1917.
14. WAKSMAN, SELMAN A. Soil fungi and their activities. Soil Sci. 2: 103–155. 1916.
15. ———. Three decades with soil fungi. Soil Sci. 58: 89–114. 1944.
16. ZELLER, S. M. Verticillium wilt of cane fruits. Oregon Agr. Exp. Sta. Bul. 344. 1936.

SOME INTERRELATIONSHIPS OF VIRUS STRAINS IN SUGAR-BEET CURLY TOP

N. J. GIDDINGS[1]

(Accepted for publication October 25, 1949)

In 1925 Carsner (2) reported that sugar-beet (*Beta vulgaris* L.) plants infected by a less virulent form of the curly-top virus, *Ruga verrucosans* Carsner and Bennett, could be infected with a more virulent form, and would then develop severe symptoms. In the same article he reported experiments which indicated that the more virulent form of virus was attenuated by passage through certain hosts. Curly-top virus attenuation was also reported by Carsner and Stahl (4), Carsner and Lackey (3), and Lackey (9, 10).

Restoration of virulence was reported by Lackey (10, 11). Giddings' (6) experiments indicated that the reported attenuation of curly-top virus was probably a separation of virus strains and that the reported restoration of virulence was a reappearance of the more virulent strain in a dominant form after being latent in the infected plants. Johnson (8) stated that attenuation, as reported by Carsner, had been disproved by Giddings (6), but in his own work with strains of tobacco mosaic virus he speaks of attenuation by means of strain separation. "Attenuation" properly connotes a weakening by change and not a separation. That is the usage followed in this article.

This paper reports evidence that none of the curly-top virus strains thus far tested will immunize against other strains of the virus. It gives data to show that one plant may be infected by at least three strains and that by proper choice of host plants, or other means, separation of these strains can be accomplished. It presents evidence that one strain may be dominant to the extent of inducing characteristic symptoms while another strain is present in the same host, and that such latent or dormant strains may later develop to induce dominant symptoms in the host.

For the convenience of readers not familiar with the different strains of curly-top virus, table 1 gives the symptoms induced by strains 1, 2, 3, and 5, the four strains most involved in the experimental work here reported.

INFECTION BY A LESS VIRULENT VIRUS STRAIN FOLLOWED BY INOCULATION WITH A MORE VIRULENT STRAIN

One of the first questions to be considered regarding the relationships of the curly-top virus strains was whether infection by one strain would im-

[1] Senior Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

munize against other strains. Sugar-beet plants infected with the less virulent curly-top strains 2, 4, or 7 were allowed to develop typical symptoms and, approximately 3 weeks after inoculation, 587 of these infected plants were reinoculated using the more virulent strains 1 and 3. More than 50 per cent of these plants developed severe symptoms within 3 weeks after inoculation, while 177 control plants which had not been inoculated with strains 1 or 3 showed gradual evidence of recovery rather than any increase in severity of symptoms. Similar results were obtained regardless of whether the first inoculation was made using just one virus or whether the

TABLE 1.—*Comparative symptoms induced in young sugar beets by the four virus strains most used in this work*

Virus strain	Symptoms on	
	Susceptible beet (S.L. 842)	Resistant beet (S.L. 68)
1	Extreme distortion, curling, and dwarfing. Occasional death.	Pronounced vein clearing, usually some leaf curling. Slight dwarfing.
3	Extreme distortion, curling, and dwarfing. Occasional death.	Infection very rare. Extremely slight vein clearing on any infected plant.
2	Either slight curling of leaves with pronounced vein clearing, or numerous papillae, or both. No obvious dwarfing.	Slight or pronounced vein clearing. Occasional slight leaf curling. No dwarfing.
5	Very little distortion, but extreme dwarfing and high mortality.	Slight vein clearing. Very rarely, slight leaf rolling. Slight dwarfing.

plants were simultaneously inoculated with strains 2 and 4 and regardless of whether strain 1, 3, 5, or 6 was used for the second inoculation. Virus was recovered from many of these plants and tests were made on differential hosts in order to learn whether both strains were to be found and whether any new or changed virus might appear. There were no evidences of changes in the virus.

INFECTION BY A HIGHLY VIRULENT STRAIN FOLLOWED BY INOCULATION WITH A LESS VIRULENT ONE

Since infection by the less virulent virus strains failed to protect sugar-beet plants against the more virulent strains, experiments were conducted to determine whether infection by a more virulent strain would protect against the less virulent. Such tests would offer no probability that infection by the second, less virulent, virus would result in any change in appearance of the severely diseased plants. The only method of proving the presence of the second virus strain is to recover it from the mixture and test it on appropriate differential hosts. Since the separation of strains 2 and 3 is very simple, these strains were used. Inoculating plants of the resistant sugar-beet variety S.L. 68 with a mixture of strains 2 and 3 results in an infection that yields only the less virulent strain 2, since strain 3 rarely infects this beet but strain 2 readily infects it. Similarly, the inoculation of plants of Turkish tobacco (*Nicotiana tabacum* L. var. Turkish) with the same virus mixture gives infected plants which yield only virus strain 3. Strain 2 does not infect it while strain 3 does.

Sugar-beet plants of the susceptible variety S.L. 842 were inoculated with strain 3 virus and a month or two later the infected plants were reinoculated using strain 2. Infection with strain 2 was easily accomplished and the two strains of virus were recovered from infected plants and identified. These tests clearly demonstrated that infection of sugar-beet plants by the highly virulent curly-top virus strain 3 did not immunize against the less virulent strain 2.

TESTS TO DETERMINE WHETHER ANY ONE OF THE FIRST SEVEN STRAINS WOULD IMMUNIZE AGAINST ANY OF THE OTHERS

As a result of the previous tests, an effort was made to determine the possible immunizing effect of each of the first seven strains of virus against each of the others in that group. There are 42 possible combinations as shown in table 2. The eighteen combinations marked (b) in this table are

TABLE 2.—*Infection of sugar-beet plants by one strain of curly-top virus subsequent to infection by any other strain of curly-top virus*

Plant originally infected by virus strain No.	Results[a] of inoculation with a second strain (strain number indicated at top of column)						
	1	2	3	4	5	6	7
1		(b)	(b)	(b)	?/13(b)	5/16	(b)
2	52/77		30/33	(b)	21/32	19/20	(b)
3	37/231	13/15		12/33	?/8(b)	?/7(b)	(b)
4	54/76	3/11	31/77		?/12(b)	12/16	(b)
5	5/11	3/5	3/3	?/14(b)		(b)	(b)
6	6/11	4/4	3/3	?/14(b)	?/7(b)		(b)
7	10/15	6/16	8/10	5/10	1/3	10/13	

[a] Number of positive infections by second strain is indicated by numerator, and number of tests by denominator. Uncertain results are indicated by a question mark (?). The (b) indicates that no satisfactory method is known for proving the presence of the second virus.

the ones in which we do not yet have a satisfactory method of proving the presence of the virus used for the second inoculation. For example, any test plant which might be infected by virus strain 2 is also readily infected by virus strain 1 and the symptoms are similar except as to severity, thus effectively blocking attempts to prove the presence of strain 2 in a possible mixture of strains 2 and 1. It doubtless would be possible to obtain strain separations by using very weak dilutions of the virus mixtures for short feedings of nonviruliferous leafhoppers and then transferring them to test plants. The dilution method of separation has not been used in this work.

This series of tests proved that infection with a second virus could be obtained in every case where a satisfactory test was known for determining the presence of the second virus in the infected plant. Seven combinations were tested in which there was no satisfactory method known for proving the presence of the second virus. It was hoped that some positive or negative evidence might be secured, but the results were indefinite and they are marked "uncertain" in table 2.

DISPARITY BETWEEN STRAINS 1 AND 3

Curly-top virus strains 3 and 1 each induce severe symptoms in susceptible sugar beets, but strain 3 rarely infects the highly resistant variety S.L. 68 while strain 1 readily infects it. Thus a susceptible plant infected by strain 3 and subsequently inoculated with strain 1 may easily be tested for the presence of the latter by feeding nonviruliferous leafhoppers on it and then transferring them to test plants of the sugar-beet variety S.L. 68. If these test plants become infected it is reasonably certain that the susceptible plant was infected by the strain 1 virus after it had been invaded by strain 3. Early attempts to superimpose strain 1 virus in susceptible beets infected by strain 3 gave negative results in ten trials. Later experiments gave 5 per cent infection (1 out of 20) in the very susceptible variety S.L. 842; 10 per cent infection (11 out of 116) in plants of sugar-beet variety U.S. 33; 20 per cent infection (11 out of 56) in the variety A 600; 23 per cent infection (18 out of 79) in variety U.S. 22; and 20 per cent infection (3 out of 15) in variety U.S. 22/3. The last three varieties are distinctly more resistant to curly top than the first two. These results suggest that the resistant varieties used had a greater inhibitory effect upon the development of strain 3 than upon strain 1, or that they contain some essential nutrient material that is more readily utilized by strain 1 than by strain 3.

TABLE 3.—*Evidence that virus strain 1 is unable to maintain itself in susceptible sugar-beet plants infected with both strains 3 and 1*

Experiment number	Date of original transfer of strains 1 and 3	Strain 1 was proved to be: Present for (months)	Absent after (months)
1	Nov. 30, 1939	7½	9½
2	Jan. 30, 1942	6	7¼
3	do	4	6
4	do	10	11¼
5	May 25, 1942	3	5¼

The disparity of strains 3 and 1 was further emphasized as a result of experiments in which leafhoppers were fed upon the two strains of virus and then maintained for long periods of time on large, susceptible sugar-beet plants. Nymphs from these leafhopper colonies were transferred to new food plants and were tested at intervals of 1 to 2 months. Both susceptible and highly resistant young sugar-beet plants were used in making the tests. The susceptible test plants would give definite evidence that virus was present or absent, and the resistant test plants would become infected only if strain 1 was present. The results of five experiments are given in table 3. Strain 1 virus was shown to be present in the mixture for periods of at least 3 months to 10 months.

Transfers of the colonies to susceptible plants were continued for some time after tests had failed to reveal any evidence of strain 1, and then the colonies used in the last four experiments were transferred to plants of the

resistant variety U.S. 22. This beet variety is far more resistant to strain 3 than to strain 1 and it was thought that strain 1 virus might reappear if any of it was still present.

In experiment No. 3 the strain 3 virus was absent from the resistant beet after 5 months and the leafhopper colony became nonviruliferous. In experiments 2, 4, and 5 the strain 3 virus was abundant after 8 months and the leafhopper colonies were transferred to plants of the highly resistant beet variety S.L. 68. The virus was absent in one of these in less than 2 months and in the others within 4 and 6 months, respectively, making all of the colonies nonviruliferous. For experiment No. 1 the leafhoppers were transferred directly from a susceptible plant to a plant of the variety S.L. 68, and strain 3 virus was absent in less than 4 months, leaving the new broods of leafhoppers nonviruliferous.

From these experiments it is evident that strain 1 virus is unable to maintain itself for a great length of time in susceptible sugar beets which are also infected with strain 3.

INOCULATIONS BY VECTORS WHICH HAVE INGESTED TWO VIRUS STRAINS

Numerous experiments have indicated that the vector carries the different strains of the virus with equal facility and some experiments were undertaken to learn the results which might be secured when the vector had ingested two known strains of virus and was then allowed to feed on susceptible sugar-beet plants.

Large nymphs which had been reared on plants infected with strain 2 virus were transferred to plants infected with strain 3 virus and allowed to feed for 16 hr. Twenty of the small leaf-cages were numbered and one nymph was placed in each cage. Each one was placed on a young, susceptible, healthy sugar-beet plant and then transferred to a similar fresh plant at the end of the feeding period. For a while (13 days) transfers were made twice daily, at about 8 A.M. and 5 P.M., so that one feeding period was 9 hr. and the next was 15 hr. Transfers were then made daily for about 3 months and then once in two days during the rest of the experiment. There were 1229 transfers to that number of test plants, and 615 of these plants became infected. One hundred and eighty-four plants (30 per cent) showed symptoms characteristic of those produced by strain 3 virus, while 431 had symptoms characteristic of strain 2. Table 4 indicates the symptoms which appeared in the first five plants infected and the last five plants infected by each of 10 of these leafhoppers.

Among the first five infected plants in the entire group used for these tests, 49 per cent showed strain 3 symptoms; among the last five infected plants, only 6 per cent showed such symptoms.

Considering the first one-fourth of the total number of transfers of each leafhopper, there were 127 infected plants of which 63 (50 per cent) showed symptoms suggesting strain 3, and 64 had symptoms characteristic

TABLE 4.—*Symptoms induced in first five and in last five test plants (young, susceptible sugar beets) that were infected by each leafhopper*

Leafhopper No.	First five infections					Last five infections				
	Symptoms were characteristic of virus strain number:									
1	2	3	3	3	3	2	2	2	2	2
2	2	2	2	2	2	2	2	2	2	2
3	2	2	2	2	2	2	2	3	2	2
6	3	3	3	2	3	2	3	2	2	2
7	2	3	3	3	2	2	2	2	2	2
10	2	3	2	3	3	2	2	2	2	2
11	2	2	3	2	3	2	2	3	2	2
12	2	3	2	2	2	2	2	2	3	2
13	3	3	2	3	2	2	2	2	2	2
16	2	3	3	2	2	2	2	2	2	2

of strain 2. A similar consideration of the last one-fourth of the transfers for each leafhopper shows 125 infections with 11 (9 per cent) having strain 3 symptoms and 114 having strain 2 symptoms. This is a very clear indication that most or all of the plants infected by strain 3 during the earlier part of the experiment were also infected by strain 2 and, in fact, that virus strain was recovered from a number of such plants.

Since a mixture of virus strains 2 and 3 may so readily exist together in the same sugar beet and since a high percentage of the plants used in this experiment were evidently infected by both strains, it seemed desirable to try to learn whether the more virulent strain 3 might be present in some of the plants which showed strain 2 symptoms, and whether strain 2 might be temporarily dominant in a plant containing both virus strains. Fourteen of the 615 infected test plants showed symptom changes strongly suggesting that strain 2 was dominant for a while and that strain 3 then became dominant (Table 5). Usually a plant developing strain 3 symptoms will give evidence of that fact within 10 days to 2 weeks after appearance of the first symptoms, as indicated by the changes in the two control plants listed in table 5.

Susceptible sugar-beet plants infected with strain 2 virus usually show some evidence of recovery within 3 or 4 weeks after infection, while an in-

TABLE 5.—*Temporary dominance of strain 2 virus followed by dominance of strain 3, as indicated by changes in severity of symptoms on some test plants*

Test plant number	From leafhopper number	Successive readings of test plant at approximately weekly intervals. Symptom grades of 1 to 2 or 2+ indicate strain 2, while grades of 3 to 5 indicate strain 3					
		1st week	2nd week	3rd week	4th week	5th week	6th week
		Grade	*Grade*	*Grade*	*Grade*	*Grade*	*Grade*
5	7	1	2	2	4	5	
5	20	1	2	2	2+	4	
30	2	–	2	2	2	3	
68	3	–	–	2	3	3	3+
Control	(Str. 3 only)	1	2	3	5	5	
Do	do	1	4	4	5	5	

crease in severity of symptoms never occurs in such plants. The first four plants indicated in table 5 show a definite or, in some cases, a pronounced increase in severity of symptoms after 3 or 4 weeks. This is good evidence that strain 2 virus was dominant in those plants during the first 2 or 3 weeks after infection, and that strain 3 virus then began to increase and soon made its presence obvious by the change in symptoms. Tests from a number of these plants showed that the symptom changes were due to the presence of strain 3 virus. To check the possible presence of virus strain 3 in test plants showing only strain 2 symptoms, 248 of these plants that were graded 2+ or less at their final reading were tested by placing nonviruliferous leafhoppers on each plant for a week, then transferring a single leafhopper to each of twelve young, susceptible sugar-beet plants and allowing them to feed on the test plant for a week. Strain 3 symptoms were developed by the secondary test plants from 35 (14 per cent) of the original ones. The final readings on these 35 original source plants were 2+ for six, 2 for twenty-one, and 1+ for eight, which shows that recovery of the strain 3 virus was not limited to those plants having the most severe symptoms.

In another experiment, young adult leafhoppers reared on a plant infected by strain 2 virus were fed for 6 hr. on a plant infected by strain 3 virus and 24 of these were placed, singly, in the small, numbered leaf-cages. Each was transferred daily to a fresh, healthy, young, susceptible sugar-beet plant. There were 1532 transfers and 1059 of the plants became infected. Strain 3 symptoms were evident in 562 plants, while the remaining 497 had strain 2 symptoms. At least 17 of the infected plants had strain 2 symptoms for some time and then developed strain 3 symptoms, indicating that the strain 2 had been dominant for a while but that strain 3 eventually began to multiply and make its presence evident. Tests from 335 of the plants showing strain 2 symptoms revealed that 60 (18 per cent) of them had strain 3 virus in sufficient amount so that typical symptoms of that strain were produced in some or all of the 12 secondary test plants. A further test from 25 of the 60 plants, using the highly resistant sugar beet S.L. 68, gave proof that strain 2 was also present in each case.

Nonviruliferous leafhoppers were allowed to feed overnight, at relatively low temperature, on a sugar-beet plant infected with strain 2 virus, and then were fed 6 hr. during the day, at relatively warm temperature, on a plant infected with strain 3 virus. They were then placed in the small, numbered leaf-cages and transferred daily to fresh, young, susceptible sugar-beet plants. There were 1350 transfers and 830 plants became infected. Strain 3 symptoms were developed by 775 plants and strain 2 symptoms by 55 plants. At least 14 of the 830 infected plants developed strain 3 symptoms after they had shown typical strain 2 symptoms for 2 weeks or more, indicating that the plant was thoroughly pervaded by the strain 2 virus before the strain 3 virus had increased to any great extent. Tests from 16 of the plants showing strain 2 symptoms at their final reading showed that strain 3 virus was present in 3 (19 per cent) of them.

It is interesting that, in the two previous experiments, the leafhoppers reared on strain 2 infected plants gave approximately 70 per cent and 46 per cent, respectively, of the infected test plants with strain 2 symptoms, while those fed overnight on a strain 2 infected plant gave only 7 per cent of the infected test plants showing strain 2 symptoms. It is also noteworthy that, in the first experiment, 35 (14 per cent) showing strain 2 symptoms at the final reading gave strain 3 virus upon further testing; in the second experiment, 60 (18 per cent); and in the third experiment, 3 (19 per cent).

INOCULATIONS BY VECTORS THAT HAVE INGESTED THREE VIRUS STRAINS

A group of young adult leafhoppers and large nymphs were fed 5 hr. on a plant infected with strain 3 virus, 5 hr. on a plant infected with strain 5 virus, and 6½ hr. on a plant infected with strain 2 virus. Twenty of these were then placed, singly, in the small leaf-cages and each one was transferred daily to a young, susceptible sugar-beet plant. There were 1038 transfers which resulted in 607 infected plants. Table 6 indicates the results which were secured with one of the leafhoppers. This illustrates the frequency of infection and the variation in symptoms developed by test plants.

TABLE 6.—*Virus strain numbers indicated by symptoms induced in test plants used for daily transfers of leafhopper No. 19 that had fed on virus strains 2, 3, and 5*

First week	0[a]	5	3	0	0	3	3
Second week	–[a]	3	0	0	0	3[b]	0
Third week	3	0	0	2	2	3	5
Fourth week	2	5	0	2	5	3	3
Fifth week	0	5	3	0	0	2	5
Sixth week	3	0	0	0	0	5	2
Seventh week	0	3	0	2	0		

[a] The 0 indicates that the plant was not infected and – means that there was no transfer made on that day.

[b] This plant had peculiar symptoms which were apparently a mixture of the characteristics of strains 3 and 5.

Leafhopper No. 19 was transferred 46 times and infected 26 plants. Seven of the infected plants showed symptoms characteristic of strain 2, 12 developed symptoms of strain 3, and seven developed symptoms of strain 5. Four times during the experiment three consecutively infected plants had symptoms which would characterize them as distinct examples of infection by the three virus strains, 2, 3, and 5, respectively. This happened to plants 5, 6, and 7 of the third week; plants 4, 5, and 6 of the fourth week; 2, 3, and 6 of the fifth week; 6 and 7 of the sixth week, and 2 of the seventh week. It is highly improbable that any of these plants were inoculated with just one virus strain, but it is clearly evident that one strain did become dominant at such a time and in such a way as to induce characteristic symptoms.

In the entire experiment, mild symptoms of strain 2 were predominant

in 299 plants; severe symptoms of strain 3 were predominant in 196 plants; and strain 5 symptoms were predominant in 112 plants. At least 15 of the 607 infected plants developed strain 3 symptoms after they had had strain 2 symptoms for 2 weeks or more, indicating that strain 2 virus had become well established in them long before strain 3 increased to any appreciable extent.

Tests were made from each of 165 infected plants to learn whether more than one strain of curly-top virus could be recovered. Each of these plants thus became a source plant from which virus was obtained and, as in previous experiments, at least 12 young, susceptible sugar-beet plants were used for this secondary test. In several cases young plants of the resistant sugar-beet variety S.L. 68 were also used in order to confirm the identification of virus strain 2.

Symptoms characteristic of virus strain 2 were evident on 103 of the source plants selected. The tests revealed that virus strains 2, 3, and 5 had developed to a sufficient amount in two of the source plants so that the virus recovered from each of these plants induced dominant symptoms characteristic of strain 2 in some of the 12 test plants, symptoms characteristic

TABLE 7.—*Evidence that dominant symptoms characteristic of one virus strain do not prove the absence of other strains in the same plant. (Inoculations were made with a mixture of strains 2, 3, and 5)*

Virus strain number as indicated by symptoms on source plant	Total source plants tested	Source plants yielding virus strain number:						
		2	3	5	2 and 3	2 and 5	3 and 5	2, 3, and 5
	No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
2	103	54	6	2	5	26	4	2
3	26	0	50	0	38	4	0	8
5	36	11	3	36	3	8	36	3

of strain 3 in others, and symptoms characteristic of strain 5 in still others. Four source plants yielded virus that induced strain 3 symptoms in some of the test plants, while others developed strain 5 symptoms. Virus from each of 27 source plants gave some infected plants showing strain 2 symptoms and others showing strain 5 symptoms. Virus from five source plants gave some infections with typical strain 2 symptoms and some with strain 3 symptoms. Two source plants yielded virus which induced only strain 5 symptoms in the test plants. Virus from each of seven source plants induced only strain 3 symptoms in the test plants. Virus from each of 56 source plants induced only strain 2 symptoms in the test plants. The first line of figures in table 7 gives the results from source plants that showed strain 2 symptoms; the second line gives similar data about source plants that showed strain 3 symptoms; and the last line gives data from source plants that showed strain 5 symptoms.

It is evident from this and the other experiments that two or more virus

strains may commonly occur in sugar-beet plants. When transfers are made from such a plant it is probable that, in most cases, all strains are included but some one of these strains generally becomes predominant, at least temporarily.

DISCUSSION

This paper presents ample evidence in support of Carsner's report that a sugar-beet plant showing mild curly-top symptoms was reinfected and with a more virulent form of the curly-top virus. It is a more surprising fact that plants infected by a highly virulent virus strain may be reinfected by a less virulent strain. Using virus strains 1 to 7 in combinations of two, it was possible to obtain infection with one and then reinfect with the other in every case where there was a practicable method known for proving that the second virus was present. Virus strains 3 and 1 present an interesting problem, since susceptible plants infected by strain 3 were difficult to infect with strain 1, while resistant plants infected by strain 3 could be reinfected with strain 1 without much trouble. The fact that strain 1 does infect is proof that strain 3 does not immunize the host against it. In susceptible beets already infected by strain 3, the apparent resistance to infection by strain 1 might be accounted for on the basis of competition for nutrients or materials essential for multiplication; but such an explanation does not hold in the case of resistant beets infected by strain 3, since strain 1 infects them more easily than it does the susceptible beet. This greater ease of reinfection in the more resistant beet could be accounted for on the assumption that there exists any one or a combination of the following factors: Strain 1 may be able to utilize some source of nutrient materials which strain 3 is unable to utilize. Strain 1 may be more efficient than strain 3 in the utilization of some nutrient source. Strain 1 may be less sensitive than strain 3 to some virus-inhibitory substance in the resistant beet. Any one of these assumptions would also account for the fact that resistant beets are more susceptible to infection and to injury by strain 1 than by strain 3. If the presence of an inhibitory substance is a major factor it might well be that both the quantity and the quality of the available virus nutrient supply is approximately the same in the resistant and the susceptible sugar-beet plants. It is certain that the members of the curly-top virus complex thus far studied do not show immunizing effects similar to those reported for various mosaic viruses.

Freitag (5) and Bennett and Wallace (1) have reported evidence that the virus does not multiply in the vector, and this was strongly confirmed as an incidental result of the experiments involving strains 2 and 3. In the first experiment using leafhoppers fed on strains 2 and 3, the first one-fourth of the transfers for each leafhopper totalled 307 of which 127 plants were infected and 63 (50 per cent) developed strain 3 symptoms, while the last 307 transfers resulted in 125 infections with only 11 (9 per cent) showing strain 3 symptoms. The data in table 4 give highly significant

evidence on this point. On the 123rd day of the experiment three of the leafhoppers were again fed on a strain 3 infected plant for about 6 hr. to see whether there would be an increase in the percentage of plants developing strain 3 symptoms. Considering an equal number of transfers for each leafhopper before this feeding and after it, the results showed that strain 3 symptoms were developed in 13 per cent of the infected plants previous to this second feeding and in 67 per cent of the plants after that feeding. This indicates that the strain 3 virus content of the leafhoppers was greatly increased by the second feeding and that the decrease in percentage of such plants previous to the 123rd day was not due to aging of the leafhopper or any such factor. Similar but less striking results were obtained from other experiments.

The many instances in which the less virulent strain 2 virus was separated from severely diseased plants which had been inoculated with a mixture of strains 2 and 3 probably explain the reported attenuation (2, 3, 9, 10) of the highly virulent virus. When virus from a severely diseased plant is passed through a resistant host and a virus is recovered which produces only mild symptoms, it would be very reasonable to assume that there had been attenuation but it may well have been merely the separation of a strain by means of differential hosts.

Greatly delayed appearance of symptoms has occurred many times in connection with various experiments. The experiments reported in this paper give further evidence along that line and the following statements of fact from them probably explain the reports of restoration (10, 11) of virulence. It has been clearly shown that a plant inoculated with both strains 2 and 3 may develop strain 2 symptoms in a few days and carry those symptoms for 2 or 3 weeks without appreciable change but may then suddenly develop the more severe strain 3 symptoms. In such cases it is clear that both strains of the virus were inoculated into the host but that strain 2 multiplied rapidly and spread throughout the plant while strain 3 remained comparatively inactive until, under some combination of favorable conditions, it also started rapid multiplication with the result that strain 2 symptoms were soon masked. It has also been demonstrated that strain 3 virus may be recovered from plants which had been inoculated with a mixture of strains 2 and 3 but which had developed only symptoms of strain 2.

A high degree of stability has been demonstrated (7) for the virus strains which have been described. This certainly does not imply that mutations never occur, but it does suggest that they are not a common occurrence under what might be considered normal conditions.

The general results obtained from the curly-top virus strain experiments are comparable with those reported by Johnson (8) for tobacco mosaic virus and by Norris (12) for the tomato spotted wilt virus.

SUMMARY

Of the seven curly-top virus strains tested there was no evidence that any one would immunize the host against infection by one of the other strains.

Recovery of the less virulent virus from plants inoculated with a virus mixture and showing symptoms of the more virulent strain indicates that this may explain previously reported cases of attenuation.

Inoculation of susceptible sugar-beet plants with a mixture of less virulent and more virulent virus strains sometimes induced only mild symptoms, but the more virulent strain could be recovered from such plants in some cases. This may explain reported cases of restoration of virulence.

Feeding a beet leafhopper on two or three distinct virus strains and then transferring it daily to a young, fresh, susceptible sugar-beet plant gave some plants that developed dominant symptoms characteristic of one strain, while others developed symptoms characteristic of another strain. The fact that both or all three of the virus strains might be present was demonstrated by recovering the virus from some of these plants and inoculating it into differential hosts. There was also clear evidence that plants inoculated with such virus mixtures might develop typical symptoms of the less virulent strain, retain those symptoms for 2 or 3 weeks, and then develop severe symptoms characteristic of the more virulent strain.

U. S. DEPARTMENT OF AGRICULTURE
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. BENNETT, C. W., and HUGH E. WALLACE. Relation of the curly-top virus to the vector. Jour. Agr. Res. [U. S.] **56**: 31–52. 1938.
2. CARSNER, EUBANKS. Attenuation of the virus of sugar beet curly top. Phytopath. **15**: 745–757. 1925.
3. ———, and C. F. LACKEY. Further studies on attenuation of sugar beet curly top. (Abstr.) Phytopath. **18**: 951. 1928.
4. ———, and C. F. STAHL. Studies on curly top disease of the sugar beet. Jour. Agr. Res. [U.S.] **28**: 297–320. 1924.
5. FREITAG, JULIUS H. Negative evidence on multiplication of curly-top virus in the beet leafhopper, *Eutettix tenellus*. Hilgardia **10**: 305–342. 1936.
6. GIDDINGS, N. J. Curly-top virus strains. (Abstr.) Phytopath. **30**: 786. 1940.
7. ———. Additional strains of the sugar beet curly-top virus. Jour. Agr. Res. [U.S.] **69**: 149–157. 1944.
8. JOHNSON, JAMES. Virus attenuation and the separation of strains by specific hosts. Phytopath. **37**: 822–837. 1947.
9. LACKEY, C. F. Attenuation of curly-top virus by resistant sugar beets which are symptomless carriers. Phytopath. **19**: 975–977. 1929.
10. ———. Further studies of the modification of sugar beet curly-top virus by its various hosts. (Abstr.) Phytopath. **19**: 1141. 1929.
11. ———. Virulence of curly-top virus restored by *Stellaria media*. (Abstr.) Phytopath. **21**: 123–124. 1931.
12. NORRIS, D. O. The strain complex and symptom variability of tomato spotted wilt virus. Coun. Sci. Ind. Res. (Aust.) Bul. 202. 1946.

PHYTOPATHOLOGICAL NOTES

A Modification of the van Tiegham Cell for Purification of Contaminated Fungus Cultures. PETER A. ARK AND ROBERT S. DICKEY. The isolation in pure culture of such fungi as *Aphanomyces, Fusarium, Pythium,* and *Saprolegnia* sometimes is difficult because of bacterial contamination. Numerous methods, including the use of the van Tiegham cell, have been mentioned in the literature to overcome this difficulty. A modification of the method described by Raper[1] has been found useful.

The method requires micro slide rings (22 mm. × 9 mm.) or van Tiegham rings, plastic modeling clay[2] (A in figure 1), Petri dishes, and suitable agar. Two or three small round pellets of clay are molded (2–3 mm. in diameter) and placed on one end of the micro slide ring (B in figure 1). The ring is

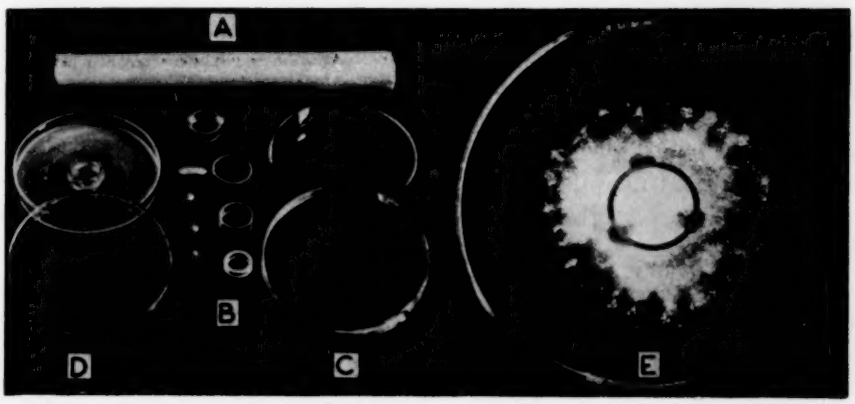

FIG. 1. A. Roll of plastic modeling clay. B. Micro slide rings and round pellets of clay. C. Petri dish. D. A full assembly in a Petri dish with the agar poured. E. Purified mycelium of *Pythium ultimum* on the outside of the ring. Material inside the ring was heavily contaminated with bacteria.

then placed in the bottom part of a Petri dish, with the pellets touching the glass. The ring is pressed gently until a clearance of about 2 mm. from the bottom is obtained. The cover is replaced and the entire apparatus sterilized in either a hot-air oven or an autoclave. After sterilization, a melted medium suitable for growth of the fungi under investigation is cooled and poured so as to fill the Petri dish and the ring to within about 3–5 mm. from the upper edge of the inside of the ring (D in figure 1). After the agar has cooled, a small piece of the contaminated material is placed on the agar in the middle of the ring. The fungus hyphae tend to grow into the agar and come out from under the ring to the surface of the agar outside the ring, free from any bacteria (E in figure 1). If there is danger of aerial mycelium growing over the edge of the ring, the agar can be acidified with

[1] Raper, John R. A method of freeing fungi from bacterial contamination. Science 85: 342. 1937.

[2] The authors used Plasti-Klay made by Schwabacher-Frey Company.

a few drops of 25 per cent lactic acid to retard the bacterial growth. One advantage of this method is that the ring is held in position by the agar. Several rings can be mounted in one plate if desired. The agar holds the ring firmly in place so plates may be inverted safely. The clay is not changed by heating and has proved nontoxic to such fungi as *Aphanomyces* sp., *Fusarium oxysporum* f. *cucurbitae*, *Pythium ultimum*, *Phytophthora capsici*, *Rhizoctonia solani*, *Verticillium albo-atrum*, and others.—Division of Plant Pathology, University of California, Berkeley 4, California.

A Technique for Observing Tuber Enlargement and Scab Development in Potatoes.[1] W. J. HOOKER.[2] A suitable technique for growing potato plants under controlled conditions which would permit daily observation of the tubers is highly desirable both for the physiologist and morphologist in studying tuberization, and for the pathologist in studying the initiation and development of tuber diseases. In order to investigate further the relationship existing between tuber enlargement, scab susceptibility, and scab lesion expansion the following culture method proved satisfactory. It is described because of its possible usefulness to other investigators concerned with various phases of potato tuber development and pathology.

Sprouted seed pieces were grown from treated tubers planted at least 4 in. deep in quartz sand. When the sprouts had emerged the best sprout was retained and the others removed. A modified 8-in. clay pot was prepared in which a 3-in. circular hole had been cut in the side with the center of the hole approximately 3 in. from the bottom of the pot (Fig. 1). The pot was inverted and placed in a 10-in. clay saucer, and peat moss was packed around the joint. The pot was then filled with sterile quartz sand to within 1 in. of the lower edge of the lateral hole. The sprouted seed piece which had been inserted through the lateral hole was partially buried in the sand and the tip of the sprout was fitted through the usual drainage hole. Peat moss was used to plug the 3-in. hole and a layer of moss was tied over the surface of the pot. The entire moss surface was maintained moist using an atomizer type sprayer twice a day. Once a week the large plug was removed and an excess of modified Hoagland's solution[3] was poured over the surface of the sand. Tuber enlargement was observed simply by removing the peat moss plug in the side of the pot, and photographs were made when desired. Tuber development was normal with no hypertrophied lenticels, and there was very little seed piece decay. The air temperature within the pot was 18°–20° C. Tuber yields were approximately equal to those generally obtained in soil culture under similar greenhouse conditions. Developing potato tubers were sprayed with a spore and mycelial suspension of *Streptomyces scabies* (Thaxt.) Waksman and Henrici, and measurements of tuber diameter and scab lesion size were made at appropriate intervals.

[1] Journal Paper No. J1711 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 816.

[2] Research Assistant Professor of the Iowa Agricultural Experiment Station.

[3] Smith, Paul G., and J. C. Walker. Certain environmental and nutritional factors affecting Aphanomyces root rot of garden pea. Jour. Agr. Res. [U.S.] 63: 1–20. 1941.

The advantages of the method described were: tuber enlargement and lesion development could be followed at daily intervals; the soil factor was largely removed; unwounded tuber surfaces could be sprayed with inoculum when desired and the interval between inoculation and lesion initiation could be determined accurately. Scab inoculations were successful and lesions were apparently normal. Further data on the relationship between active tuber enlargement and scab susceptibility were obtained.—Iowa Agricultural Experiment Station, Ames, Iowa.

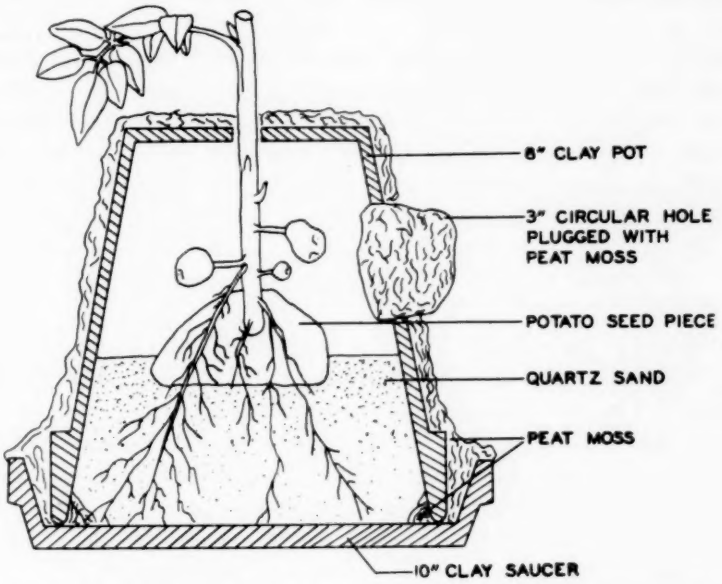

FIG. 1. Inverted pot arrangement for observing potato tuber enlargement and scab development after spraying immature tubers with *Streptomyces scabies* inoculum.

Natural Occurrence of Tobacco Necrosis Virus in Primrose.[1] W. C. PRICE, FRANK P. MCWHORTER, AND BETTY H. STERANKA. Tobacco necrosis virus is present in greenhouses in the British Isles and in Australia.[2] While usually confined to root systems of its hosts or to leaves actually in contact with the soil, it has been found also in the foliage of healthy-appearing plants of *Primula obconica* Hance.[3] This note constitutes the first report of the virus occurring naturally in any host in the United States. It also confirms Bawden's[3] observation that the virus occurs in the foliage of *Primula* species.

Our February 1948 survey of plants of *Primula malacoides* Franch in the Phipps Conservatory, Pittsburgh, Pennsylvania, revealed many plants with various sorts of mottling and chlorosis. The symptoms were similar

[1] Contribution from the Plant Virus Laboratory of the University of Pittsburgh.

[2] Smith, K. M., and J. G. Bald. A description of a necrotic virus disease affecting tobacco and other plants. Parasitology **27**: 231–245. 1935.

[3] Bawden, F. C., and B. Kassanis. Primula obconica, a carrier of tobacco necrosis virus. Ann. App. Biol. **34**: 127–135. 1947.

to those produced on primrose plants in Oregon by a strain of cucumber mosaic virus. An attempt was made to transfer the virus by the carborundum method to the cotyledons of very young cucumber seedlings. Transmission was obtained from only one of the primrose plants. Symptoms in cucumber consisted of a few small, white, sunken lesions which enlarged to well-defined spots about 4 mm. in diameter, with brown margins. Subinoculations from these lesions to cucumber and other plants produced numerous necrotic local lesions resembling those caused by tobacco necrosis virus in the same species of plants. The virus was not cucumber mosaic virus.

Since subsequent studies showed this virus from primrose to have properties and host range similar to those of tobacco necrosis virus,[4, 5] a second attempt was made in February, 1949, to find it occurring naturally in primroses. The foliage of each of approximately 100 *Primula malacoides* and 50 *P. obconica* plants was tested separately by subinoculation to young cucumber seedlings. Most of the primrose plants showed various degrees of chlorosis. A virus was obtained from only one of the plants tested, namely a plant of *P. obconica*. It was identical in all respects with the one isolated in 1948.

The thermal inactivation point of the primrose virus was found to be above 85° C. Samples heated for 10 min. at this temperature produced an average of 30 local lesions per leaf on 10 leaves of Turkish tobacco plants. The virus was infectious after drying for 11 days in leaves of Early Garden Golden Cluster bean plants. It produced a localized infection in all susceptible plants tested, including *Primula malacoides*, *Coleus blumei* Benth., *Cucumis sativus* L., *Nicotiana glutinosa* L., *N. rustica* L., *N. sylvestris* Spegaz. and Comes, *N. tabacum* L., *Phaseolus vulgaris* L., *Salpiglossis sinuata* Ruiz. and Pav., *Sinningia speciosa* Benth. and Hook., *Vinca rosea* L., and *Viola cornuta* L. The species *Impatiens balsamina* L. and *Begonia semperflorens* Link and Otto proved insusceptible.

Thus, the host range and properties of the virus from primrose, in so far as tested, proved identical with those of tobacco necrosis virus. There was no reason to believe that this virus accounted for any of the mottling or chlorosis of the primroses. These findings are in agreement with those reported by Bawden[3] except that the Pittsburgh isolate did produce visible localized infection in *Primula malacoides*.—Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pa.

The Effect of Yellow Dwarf on Yield of Onion Seed.[1] R. D. WATSON AND GLENN KENKNIGHT.[2] Yellow dwarf has been unimportant on the

[4] Price, W. C. Studies on the virus of tobacco necrosis. Amer. Jour. Bot. 25: 603–612. 1938.

[5] Price, W. C. Comparative host range of six plant viruses. Amer. Jour. Bot. 27: 530–541. 1940.

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 291.

[2] Associate Plant Pathologist and former Associate Plant Pathologist, respectively, Idaho Agricultural Experiment Station, Moscow, Idaho.

commercial onion bulb crop in Idaho. In the onion seed crop, however, it has at times caused heavy losses. Little information is available on the nature and degree of losses caused by plant diseases in onion or other vegetable seed crops. Losses to the seed crop caused by yellow dwarf were studied in a field of Ebenezer onions.

Onion seed heads were collected from individual plants having yellow dwarf and those not having yellow dwarf, here referred to as "healthy." Some other diseases in the field, such as bulb rots and pink root, were assumed to be equally prevalent on yellow dwarf and healthy plants.

Two rows were selected at random in each of three parts of the field. From each row the first 10 healthy individual plants and from the first 10 individuals infected with yellow dwarf, seed heads were collected, threshed, and cleaned in water. The seed were dried and the seed weights from the individual onion plants were obtained. The center part (B in table 1) of

TABLE 1.—*Effect of yellow dwarf on seed yield of Ebenezer onions*

Portion of field sampled	Mean weight of seed per plant in grams	
	Yellow dwarf plants	Healthy plants
A. First seed crop	1.088	2.889
B. Second seed crop	0.690	2.246
C. First seed crop	0.987	2.721
Mean of totals	0.922	2.625

Difference required for significance: 19: 1, 0.306; 99: 1, 0.404.

the field had produced a seed crop the year before and the parts (A and C in table 1) on each side were developing their first seed crop. Analysis of variance and some comparisons were made by correlation on these data.

Onion plants infected with yellow dwarf yield about one-third as much seed as plants not infected with this disease (Table 1). Other than the significant reduction in seed yield from yellow-dwarf plants there were no differences observed in quality of the seed as judged by percentage germination, seedling weight, or apparent vigor of the seedlings.

The part of the field developing the second seed crop yielded less seed than the portions on either side. Yellow dwarf in the second seed crop reduced yields more than it did on the two first seed crops.

Yellow dwarf affected the vigor and growth of the plant as indicated by the length of the seed stalk. On infected plants only 6.8 per cent of the seed stalks were over 36 in. long, whereas on healthy plants 86.2 per cent of the seed stalks were over 36 in. long. As a whole, the significant correlation of these data was 0.81 between high seed yield and long length of seed stalk.—Idaho Agriculture Experiment Station, Moscow, Idaho.

REPORT OF THE 41ST ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its forty-first annual meeting at the Hotel Martinique, New York, N. Y., on December 28, 29, and 30, 1949. The Northeastern Division met with the parent Society. Business meetings were held on December 28 and 30. The Council met on December 27 and again on the 28th. On the afternoon of December 30, a joint session was held with the Mycological Society of America. Four hundred and twenty-five members registered. One hundred and sixty-two papers were scheduled in fourteen sessions, and four symposia. Conferences and symposia were held under the following titles: Stone Fruit Virus Diseases, Extension Work in Plant Pathology, The Teaching of Plant Pathology, Fungicide Colloquium, Plant Disease Forecasting, Performance of New Fungicidal Sprays and Dusts in Cooperative Regional Experiments Conducted in 1949, and Outlook for Cooperative Regional Projects in Plant Pathology under the Research and Marketing Act. A trip was scheduled to the Foreign Plant Quarantine Inspection House of the U. S. Department of Agriculture at Hoboken, New Jersey. Inspection and treatment methods were observed and quarantine problems discussed. One hundred and forty-nine applicants were accepted as members of the Society.

The annual dinner at the Hotel McAlpin was attended by approximately 250 members and guests. Entertainment was provided through the generous contributions of friends of the Society.

The AAAS $1,000 Prize was awarded to Dr. Armin C. Braun, author of the paper "Thermal inactivation studies on the tumor-inducing principle in crown gall," presented on the program of The American Phytopathological Society.

Council for 1950:

C. M. TUCKER, President (1 yr.), University of Missouri, Columbia, Missouri
J. G. HORSFALL, Vice-President (1 yr.), Connecticut Agricultural Experiment Station, New Haven 4, Connecticut
CURTIS MAY, Secretary (3-yr. term expires December, 1950), Plant Industry Station, Beltsville, Maryland
A. E. DIMOND, Treasurer of the Society and Business Manager of PHYTOPATHOLOGY (3-yr. term expires 1952), Connecticut Agricultural Experiment Station, P.O. Drawer 1106, New Haven 4, Connecticut
HELEN HART, Editor-in-Chief of PHYTOPATHOLOGY (3-yr. term expires 1951), University Farm, St. Paul 1, Minnesota
W. D. VALLEAU, President 1949, Kentucky Agricultural Experiment Station, Lexington 29, Kentucky
W. H. TISDALE, Councilor-at-large (2-yr. term expires December, 1950), Du Pont Experiment Station, Wilmington 98, Delaware
GEORGE L. MCNEW, Councilor-at-large (2-yr. term expires 1951), Boyce Thompson Institute for Plant Research, Yonkers, New York
PAUL R. MILLER, Potomac Division, Plant Industry Station, Beltsville, Maryland
A. L. SMITH, Southern Division, University of Alabama, Auburn, Alabama
M. T. HILBORN, Northeastern Division, University of Maine, Orono, Maine
W. F. BUCHHOLTZ, North Central Division, Iowa State College, Ames, Iowa
L. C. COCHRAN, Pacific Division, Citrus Experiment Station, U. S. Department of Agriculture, Riverside, California

Representatives:

AAAS Council. R. S. KIRBY (1949–50), H. C. YOUNG, SR. (1950–51)
Division of Biology and Agriculture, National Research Council. J. C. WALKER (1950)
American Institute of Biological Sciences. J. C. WALKER (4-yr. term expires June 30, 1954.)
Board of Editors, American Journal of Botany. C. C. CHUPP
American Type Culture Collection. H. H. MCKINNEY
Scientific Liaison and Advisory Board, Quartermaster Food and Container Institute for the Armed Forces. H. H. THORNBERRY
Sixth International Botanical Congress, Nomenclature Section, Stockholm, Sweden, 1950. J. A. STEVENSON

Standing Committees:

Donations and Legacies: FREEMAN WEISS, Chm.; P. P. PIRONE, A. A. DUNLAP, W. H. TISDALE, N. J. GIDDINGS

Extension: J. O. ANDES, Chm.; O. C. BOYD, H. R. GARISS, L. O. WEAVER, R. J. HASKELL, S. B. FENNE, E. G. SHARVELLE, J. E. LIVINGSTON, C. E. SCOTT, C. F. BISHOP, A. H. BAUER

Finances and Investments: M. C. RICHARDS, Chm.; A. E. DIMOND, FREEMAN WEISS, W. H. TISDALE, DONALD CATION

Phytopathological Classics: L. J. TYLER, Business Manager; L. C. KNORR, EDITOR

Placement: R. S. KIRBY, Chm.; H. A. RODENHISER, M. W. GARDNER, R. J. HASKELL, L. M. MASSEY, S. J. P. CHILTON, E. E. HONEY

Public Relations: GEORGE L. MCNEW, Chm.; PAUL R. MILLER, K. S. CHESTER, L. S. HITCHNER, J. D. MOORE, GEORGE ZENTMYER, HARRY O'BRIEN, C. E. COX, R. R. KINCAID, O. D. BURKE, I. J. CONNORS

International Cooperation: E. C. STAKMAN, Chm.; A. G. KEVORKIAN, I. E. MELHUS, M. H. LANGFORD, G. H. COONS, J. G. HARRAR, J. A. STEVENSON, OTTO A. REINKING, H. P. BARSS

Plant Disease Prevention: J. G. LEACH, Chm.; PAUL R. MILLER, S. B. FRACKER, E. C. STAKMAN, L. C. COCHRAN, H. H. THORNBERRY

Subcommittee on Regulatory Work and Foreign Plant Diseases: C. R. ORTON, Chm.; R. P. WHITE, W. H. WHEELER, E. G. REX

Subcommittee on Seed and Plant Material Certification: H. C. MURPHY, Chm.; L. C. COCHRAN, REINER BONDE, CHARLES CHUPP, C. H. ARNDT, JOHN HARDISON

Subcommittee on Utilization of Plant Pathologists and Facilities in National Emergencies: H. H. THORNBERRY, Chm.; H. T. COOK, H. P. BARSS, A. L. ANDERSON, M. F. KERNKAMP

Special Committees:

Fungicides: D. W. POWELL, Chm.; W. D. MCCLELLAN, J. G. HORSFALL, F. L. HOWARD

Subcommittee on Testing and Results of Newer Fungicides: W. D. MCCLELLAN, Chm.; M. C. GOLDSWORTHY, E. G. SHARVELLE, M. B. LINN, E. E. WILSON, R. W. LEUKEL, PAUL R. MILLER, J. D. WILSON

Subcommittee on Special Problems: F. L. HOWARD, Chm.; L. S. HITCHNER, H. C. YOUNG, SR.

Subcommittee on Fungicide Nomenclature: D. W. POWELL, Chm.; J. D. MOORE, W. H. TISDALE, J. C. DUNEGAN, E. A. WALKER, GEORGE A. ZENTMYER

Fungus Nomenclature: J. A. STEVENSON, Chm.; W. W. RAY, W. C. SNYDER, D. E. ELLIS, C. M. CHRISTENSEN

Nomenclature and Classification of Plant Viruses: W. C. PRICE, Chm.; FREEMAN WEISS, C. W. BENNETT, H. H. MCKINNEY, JAMES JOHNSON, FRANK MCWHORTER, L. C. COCHRAN

Subcommittee on Virus Type-Culture Collection: H. H. MCKINNEY, Chm.; JAMES JOHNSON, T. J. GRANT

Membership: GLENN S. POUND, Chm.; W. J. JEFFERS, B. H. DAVIS, C. N. CLAYTON, P. M. SIMMONDS, THOR KOMMEDAHL, L. K. WRIGHT, A. W. DIMOCK, E. C. TIMS, A. E. DIMOND, ex officio

Sustaining Associates: S. J. P. CHILTON, C. J. NUSBAUM, L. D. LEACH

Nomenclature of Plant Pathogenic Bacteria: O. N. ALLEN, Chm.; J. G. LEACH, W. H. BURKHOLDER, CHARLOTTE ELLIOT, E. M. HILDEBRAND, P. A. ARK

Teaching of Plant Pathology: G. C. KENT, Chm.; J. G. LEACH, THOMAS SPROSTON, M. F. KERNKAMP, G. F. WEBER

Temporary Committees for 1950:

Publication of Special Material: DONALD CATION, Chm.; H. P. BARSS, G. C. KENT, W. H. TISDALE

Spray Tolerances: GEORGE L. MCNEW, Chm.; L. M. MASSEY, H. C. YOUNG, SR., J. G. HORSFALL, J. W. HEUBERGER, PAUL R. MILLER, S. E. A. MCCALLAN, L. J. KLOTZ

Report of the Secretary. On December 22, 1949, the total membership was 1,575, consisting of 141 life members, 1,285 regular members, and 149 applicants to be accepted at the New York meeting. Reports were received of the death of five members during the year; Neil Stevens (life), B. M. Gaddis, Raymond B. Wilcox, H. M. Fitzpatrick, Caroline T. Rumbold (life). Hans W. Wollenweber, formerly a member, died during the year.

Report of the Treasurer. Statement of accounts for the year ending September 30, 1949.

Receipts:

Balance from 1948	$ 5,880.79

Annual dues and member subscriptions:			
1948	$ 182.00		
1949	7,670.27		
1950–51	1,033.75	$8,886.02	
Sales		37.00	
Sustaining Associates		200.00	
Received from PHYTOPATHOLOGY for editorial and secretarial work		233.53	
AIBS		989.50	
Miscellaneous		12.50	$10,358.55
Total receipts			$16,239.34
Expenditures:			
Member subscriptions transferred to PHYTOPATHOLOGY			
1948	$ 100.00		
1949	6,390.47		
1950–1951	823.50	$7,313.97	
Transferred to PHYTOPATHOLOGY for sales	37.00		
		$7,350.97	
Office of Editor-in-Chief			
Editorial and secretarial work		233.53	
Office of President			
Supplies and postage		15.05	
Office of Secretary			
Stamps		9.54	
Printing		289.29	
Other		7.99	
Office of Treasurer			
Secretarial work		532.45	
Audit		3.00	
Printing		26.55	
Annual Meeting			
Rooms	$49.35		
Travel	63.49	112.84	
Placement Committee		3.00	
Withholding tax		10.56	
Bonds, refund, miscellaneous		24.00	
AIBS		850.00	
Total expenditures			$ 9,468.77
Balance on hand October 30, 1949			6,770.57
			$16,239.34

M. C. RICHARDS, *Treasurer*

Report of the Business Manager. The costs of carrying out the functions of the Business Office increased this year over 1948 by $3,761.74. With 1,429 members and 931 subscribers there was a total of 2,371 copies of PHYTOPATHOLOGY distributed each month during 1949. This is 15 more than in 1948.

Statement of accounts for the year ending September 30, 1949.

Receipts:			
Balance on hand from 1948			$ 5,702.64
Subscriptions:			
1948	$ 287.10		
1949	6,083.05		
1950–1953	566.40	$6,936.55	
Member subscriptions and sales transferred from APS			
1948	$ 100.00		
1949	6,390.47		
1950–1951	823.50		
Sales	37.00	7,350.97	

Receipt to PHYTOPATHOLOGY from APS	268.72		
Sales back numbers of PHYTOPATHOLOGY	1,771.77		
Sales of membership list	13.00		
Sales of 30-Year Index	43.55		
Reprints	2,424.07		
Excess illustrations	313.28		
Rockefeller Institute contribution	600.00		
Interest on sinking funds	116.50		
Donations and Legacies fund	20.00		
Transfer to APS	8.00		
Advertising	3,895.13		
Sustaining Associates	1,300.00		
Total receipts		$25,061.54	
Bank balance 1948 and receipts for 1949			$30,764.18
Northwestern Federal Savings & Loan			5,501.94
Grand total			$36,266.12

Expenditures:

Printing, distributing and storing PHYTOPATHOLOGY			
Volume 38, No. 9	$ 991.92		
10	1,091.81		
11	1,054.21		
12	1,521.23		
Volume 39, No. 1	1,243.92		
2	1,201.46		
3	1,023.63		
4	1,415.30		
5	1,210.35		
6	1,371.65		
7	1,240.43		
8	992.96		
9	1,220.62		
Postage	1,054.45		
Engravings	1,541.21	$18,175.15	
Office of Business Manager			
Secretary	$ 619.50		
Supplies	18.22		
Stamps	92.50		
Miscellaneous	2.84	733.06	
Office of Editor-in-Chief			
Secretary	$1,110.93		
Supplies	102.96		
Express	20.94		
Miscellaneous	1.79	1,236.62	
Advertising Manager			
Secretary	$ 82.00		
Postage	13.94		
Commission	233.65		
Miscellaneous	16.90	346.49	
Purchase of back volumes		118.37	
Refunds		90.27	
Miscellaneous		268.72	
News Letter		238.06	
Printing		34.78	
Agency Commission—Advertising		59.40	

Transfer to APS	8.00	
Reprints	1,666.71	
Total expenditures		$22,975.63
Balance on hand:		
Checking account	$7,788.55	
Northwestern Federal Savings & Loan	5,501.94	13,290.49
Grand total		$36,266.12

Sinking Fund. The principal amount of the sinking fund remained the same as for the previous year.

First mortgage note, at 3.8 per cent interest, deposited with McLachlen Banking Corporation for collection	$ 500.00
U. S. Savings Bond, Series G, No. M19056026, 2½ per cent interest	1,000.00
Northwestern Federal Savings & Loan Ass'n (certificates)	2,000.00
War Savings Bonds, Series F (maturity value)	1,150.00
Jefferson Federal Savings & Loan Ass'n (accrued dividends, $121.87)	621.87
District Bldg. & Loan Association (accrued dividends, $361.25)	1,861.25
National Permanent Bldg. Ass'n (accrued dividends, $606.53)	2,606.53
Perpetual Building Association (accrued dividends, $243.78)	1,243.78
Prudential Building Association (accrued dividends, $52.44)	258.78
Arlington and Fairfax Bldg. & Loan (accrued dividends, $143.32)	1,143.32
	$12,385.53
Less interest due PHYTOPATHOLOGY	1,529.19
	$10,856.34

Lyman Memorial Fund. This fund is obtained from voluntary contributions and now totals $4,065.08. This amount is invested with the Brookland Building and Loan Association at 2½ per cent interest.

Balance on hand September 30, 1948	$3,958.94
Dividends, December 31, 1948, and June 30, 1949	99.64
Voluntary contributions	6.50
	4,065.08
Less interest due PHYTOPATHOLOGY	681.85
	$3,383.23

M. C. RICHARDS, *Business Manager*

Report of the Auditing Committee for the fiscal year, 1948–1949. We have examined the books of the Treasurer of The American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY for the period from September 30, 1948, to September 30, 1949. We found all receipts and expenditures and all funds of the Society and of PHYTOPATHOLOGY accurately recorded.

Signed: R. C. COOMBS
G. A. MCLAUGHLIN

Report of the Advertising Manager, 1949.

Number of advertisers	Space used (pages)	Gross income	Net income
104	90.0	$3,742.80	$3,265.58

Expenses: Stenographic and miscellaneous—$97.97

Pages of non-paying ads:

Biological Abstracts, ½ page 3 times	1½
Placement Committee, ½ page 3 times	1½
Phytopathological Classics, ½, 1, ½, 1, 1	4
Forms for Bequest and Codicil, ½	1½
Committee on Donations and Legacies, 1	1
Total	9

L. J. ALEXANDER, *Advertising Manager*

Report of the Editor-in-Chief. Volume 39 of Phytopathology contains 1066 pages in which 364 authors published 99 articles, 32 Phytopathological Notes, 184 abstracts, and 2 biographies. Three announcements and the annual report were published for the Society, and reports, including abstracts usually, for the Northeastern Division, the Pacific Division, the Potomac Division, and the Southern Division. Thirteen abstracts were published by title only. Between December 1, 1948, and November 30, 1949, fourteen manuscripts were withdrawn or rejected.

Manuscripts have accumulated during the last six months of 1949 and publication may require a longer time unless the size of the journal is increased. On December 1, 1949, the material on hand for 1950 was: 18 manuscripts in galley proof, 24 manuscripts in press, 22 manuscripts accepted and in editorial process, 13 manuscripts in need of authors' revisions, and 15 manuscripts submitted to reviewers.

HELEN HART, *Editor-in-Chief*

Report of the Editor of Phytopath News. During the calendar year 1949, six numbers of NEWS were published. The expenses for the fiscal year approached the $300 appropriated for publication. If printing and distribution costs do not increase appreciably and if the Society feels the expenditure is justified, $300 should provide for six numbers of NEWS in 1950.

More items are being contributed voluntarily now that the newsletter is becoming established. Notices of meetings are also being more widely publicized through NEWS. If every member utilizes the newsletter to present, as well as to glean, items of interest, the purpose for which NEWS was established will be realized.

From November 17, 1948, through August 31, 1949, publishing costs of NEWS were as follows: printing, $124.25; addressing, $67.26; postage, $61.80; total, $253.31.

K. W. KREITLOW, *Editor*

Report of Representative to the American Institute of Biological Sciences and the Division of Biology and Agriculture, National Research Council. A brief summary of the progress and actions of AIBS and NRC of particular importance to APS during the current year follows.

Distinct progress was made by the Committee Advisory to the Armed Forces in that for the first time Biology was fully represented along with Chemistry, Physics, and Engineering in conferences with the Selective Service Office. New points of view regarding conservation of scientific manpower suggested by AIBS have been favorably received at the policy-making level of the Government.

The Committee on Publication Problems (A. J. Riker, Chairman) has made distinct progress in bringing together information from editors and publishers. A symposium on uniformity of botanical citations has been arranged for the New York Meetings (December, 1949).

The Handbook of Biological Data, initiated through AIBS by a grant from the Armed Services for assemblage of tabular quantitative data on blood, has been broadened in plan to include eventually general biological data. Steps have already been taken to secure titles of tables of interest to Phytopathology, and preliminary assemblage of data is under way.

An advisory relation has been established with the Office of Naval Research, aimed toward close correlation of research objectives and contracts concerned with various fundamental and applied biological problems of interest to the Navy.

With reference to educational rehabilitation of war-devastated countries under way in UNESCO, AIBS has decided for the time being to encourage individual societies to initiate or continue international cooperation within their own respective fields.

The addressograph plates set up in AIBS are about 40 per cent completed (October, 1949). They will soon be available for use of member societies without charge.

A study of financial requirements for biological research throughout the country has been initiated to supply information when called for to the Budget Bureau in connection with the proposed National Science Foundation and other Government agencies.

The Executive Committee of AIBS has gone on record as favoring a policy regarding society dues in which the base is that of active members (not including paid-up life members) who are citizens of the United States.

J. C. WALKER

Report of Representative, Board of Editors of the American Journal of Botany. Two manuscripts were examined, namely: "The effect of pH on the linear aggregation of tobacco mosaic virus," by W. N. Takahashi; and "Plant nutrition in relation to disease development. V. Bacterial wilt of tomato," by M. E. Gallegly and J. C. Walker.

CHARLES CHUPP

Report of the Committee on Donations and Legacies. In the belief that there is little prospect at present of obtaining any substantial donations from individuals or commercial establishments that might thereby wish to become patrons of phytopathological science, this Committee felt that a direct appeal to the members of the Society for modest contributions to its endowment funds was in order. It also sought some way of making it attractive to members to contribute to the Society (aside from the payment of annual dues), by offering them something in return. This was done by using a page in the September, 1949, PHYTOPATHOLOGY to carry directly to the members a message on the needs of the Society for more adequate endowment. Members were asked to state their preference among several methods of obtaining funds for this purpose, in the hope that this Committee might thereby advise the Council accordingly. The response to this question was something less than terrific, the total number of replies being three. Through the cooperation of the Secretary, a second notice to the members of the imminence of this question was sent in the program for the New York meeting.

A direct solicitation of the members for voluntary contributions of any amount involves the least change in the Society's regular procedure, and is most likely to produce at least some income in 1950. Accordingly, we recommend that Plan III, as stated in the message previously referred to, be put into effect when the bills for dues are mailed out next year. We believe that putting this solicitation on a voluntary basis is preferable at this time to raising the dues by one dollar even as a temporary arrangement, because it allows latitude for ability to pay.

We recommend also that further study and publicity be given to Plans I and II with a view to accomplishing the additional purpose of keeping the Society in touch with its retired members.

FREEMAN WEISS, *Chairman*

Report of the Extension Committee. The main purpose of this Committee is to sponsor an Annual Extension Workers' Conference at which subjects of prime importance to extension pathologists are discussed. This conference is a component of the program of the Society's annual meetings. Another function of the Committee is to study and report upon five projects through as many subcommittees. These subprojects are: 1) the extension pathologist's bookshelf, 2) airplane dusting and spraying, 3) training for extension pathologists, 4) office filing and indexing systems, and 5) collection and distribution of Kodachrome slides and slide-films on diseases of vegetables, fruits, and cereals.

Committee activities in 1949:

1. Report of the 1948 Conference in Pittsburgh.

The newly appointed Extension Committee for 1949 prepared a mimeographed report of the Pittsburgh Conference and sent a copy to each extension pathologist in the country.

2. Meeting of the Committee in Pittsburgh before adjournment of the Society to decide upon activities for the current year. It was voted to continue the five subprojects listed above, to hold a 3- to 4-hour conference at the New York meetings in December, and to arrange an extension pathologists' luncheon before or after the conference.

3. Formulation of program for the 1949 conference: At the Committee meeting in Pittsburgh it was decided that the 1949 Conference should consist of purely informal discussions by extension pathologists of subjects of prime importance to the group; the theme of the discussion to be "What's New in Disease Control?" It was also voted to divide the discussion subjects into several groups with a leader designated for each group.

4. Canvassing extension workers for conference subjects. The Committee sent a questionnaire to all extension pathologists for a list of their preferred subjects to be discussed at the annual conference.

5. Subcommittee projects. At least some of the five leaders of subprojects have made additional headway during the current year, and all will submit reports at the annual conference. It will be recalled that in 1948, R. J. Haskell's subcommittee made it possible for extension pathologists throughout the country to obtain copies of good 2×2 Kodachrome slides on diseases of fruits, vegetables, and cereals. This year, the subcommittee arranged through the U. S. Department of Agriculture to have the best of those Kodachromes available in slidefilm form to extension pathologists, county agents, and other agricultural teachers. Each extension pathologist was furnished the series of three slidefilms, who in turn notified his county agents or vocational agricultural instructors of their availability, source and cost. The slidefilms are as follows: No. C-3, Diseases of Vegetables, 103 frames, $9.50; No. C-4, Diseases of Cereals, 81 frames, $8.50; No. C-5, Diseases of Fruits, 53 frames, $6.50.

6. Program outline for the 1949 conference. As this Committee report is being prepared, so is the printed program for the 1949 conference. When completed a copy will

be sent to each extension pathologist in order that all may know well ahead of the meetings in New York the exact nature of the conference program.

O. C. BOYD, *Chairman*

Report of the Investment Committee. Total investments of the Society for the year ending September 30, 1949:

Sinking fund:		
Cash value	$11,235.53	
Maturity value bonds	1,150.00	$12,385.53
Lyman Memorial Fund		$ 4,065.08

M. C. RICHARDS, *Chairman*

Report of the Business Manager, Phytopathological Classics. (Year ending Sept. 30, 1949.)

Classic No. 1:	Out of print.		
Classic No. 2:	On hand, October 1, 1948	147	
	Sold during year	35	$ 17.50
	On hand, Sept. 30, 1949	112	
Classic No. 3:	On hand, October 1, 1948	229	
	Sold during year	37	18.50
	On hand, Sept. 30, 1949	192	
Classic No. 4:	On hand, October 1, 1948	295	
	Sold during year	36	27.00
	On hand, Sept. 30, 1949	259	
Classic No. 5:	On hand, October 1, 1948	522	
	Sold during year	43	53.75
	On hand, Sept. 30, 1949	479	
Classic No. 6:	On hand, October 1, 1948	607	
	Sold during year	47	35.25
	On hand, Sept. 30, 1949	560	
Classic No. 7:	On hand, October 1, 1948	606	
	Sold during year	70	52.50
	On hand, Sept. 30, 1949	536	
Classic No. 8:	On hand, October 1, 1948	821	
	Sold during year	116	174.00
	On hand, Sept. 30, 1949	705	
Value of classics sent out, fiscal year 1948–49			$378.50

Money received during fiscal year 1948–49		
For Classics sent out	$347.25	
On accounts due previous years	29.25	
Walker Fertilizer Co. (intended as payment on account for PHYTOPATHOLOGY)	6.00	
	$382.50	
Due on accounts		
Previous years	$ 9.75	
Fiscal year 1948–49	31.25	
Total	$ 41.00	
Assets:		
Cash balance on Oct. 1, 1948	$284.63	
Receipts during fiscal year 1948–49	382.50	
	$667.13	$667.13
Liabilities:		
Postage for mailing out Classics and for correspondence	$ 25.00	
Cost and printing of 750 penny postcards for sales promotion	15.00	
Refund check in the amount of $6.00 to The Amer. Phytopath. Soc. for Walker Fertilizer Co. check drawn in favor of Phytopathological classics but intended as payment on account for the Journal of Phytopathology. To date, the refund check has not been returned to the bank for payment.		
	$ 40.00	40.00
Cash balance on Sept. 30, 1949		$627.13
Due on accounts Sept. 30, 1949	$ 41.00	

L. J. TYLER, *Business Manager*

Report of the Placement Committee. During the past year there developed the first slackening in demand for applications since the end of the war. Forty-five plant pathologists had applications in the active files of the Committee; prospective employers requested applications in an attempt to fill 30 positions; and 124 applications were sent to prospective employers. Incomplete records show that at least five plant pathologists obtained positions through the efforts of the Committee. During the year the application form was revised.

R. S. KIRBY, *Chairman*

Report of the Committee on Public Relations. The Committee expended considerable effort in promoting good public relations for the New York meetings. Heads of departments of botany and plant pathology in the United States were urged to see that their staff members provided popular abstracts to the press service of AAAS. A press office was established for the meetings. All metropolitan newspapers, journals, and radio stations were invited to contact these headquarters for news releases and interpretative advice on newsworthy papers. A bureau was established for providing speakers for radio, television, and newsreels, and contacts for such outlets were made through AAAS press service. Abstracts that provide historical perspective and economic interpretations are being prepared for release to the press to stimulate their interest in the papers at the annual meetings.

During the year, the Committee encouraged the release of newsworthy phytopathological achievements through popular media. Articles were prepared by the Committee or encouraged for publication in the following outlets: Agricultural Chemicals, AIF News, What's New in Crops and Soils, Farm Journal, Southern Planter, Maryland Vegetable Growers News, National Shade Tree Conference, Country Gentleman, Rohm and Haas Reporter. Extensively used press releases were provided on: forecasting disease outbreaks, the role of the common barberry in black stem rust epiphytotics, the threat of wilts and viruses to our shade trees. Manuscript for a documentary book on "Plant Disease Losses: Their Appraisal and Interpretation" was submitted to Chronica Botanica by K. S. Chester of the committee.

G. L. MCNEW, *Chairman*

Report of the Committee on International Cooperation. Following a visit by the chairman to a number of Japanese scientific institutions in 1948, a memorandum was sent by the Committee on International Cooperation to the Japanese Phytopathological Society, to be read at their meeting, offering the assistance of The American Phytopathological Society. Addresses of 10 libraries in institutions in Japan were obtained and published in NEWS for September–October, with a plea for literature for the Japanese. Publications have been sent and acknowledged from a number of departments in this country. Other war-devastated areas also have received literature. In some cases individuals have sent copies of PHYTOPATHOLOGY to individuals in Japan and other countries.

In a paper on "The Control of Plant Diseases" given on August 30 at the UN Scientific Conference on the Conservation and Utilization of Resources, at Lake Success, N. Y., the chairman of the Committee made the following statement:

"In many cases there are no natural barriers to the international dissemination of plant pathogens, and artificial barriers often are ineffective; accordingly, disease control on an adequate scale becomes a regional and often an international problem. Unfortunately, however, most plant pathologists cannot migrate as freely as many plant pathogens. This gives the pathogen a decided advantage.

"Many plant scientists long have recognized the need for international cooperation in plant improvement and plant protection and have practised it within the limits of their abilities and facilities. There are international scientific societies that do what they can to help meet some of the needs for cooperative effort, to which all too brief allusions have been made in this paper. But too often good intentions and well-conceived plans are abortive because of the lack of a definite organization whose definite function it is to capitalize intentions and implement plans.

"Recognizing the need for systematized exchange of information on plant diseases and their control, The American Phytopathological Society, through its Committee on International Cooperation, made the following proposals to plant pathologists in a number of countries to provide for the following: 1) Exchange of information and materials in connection with the development of disease-resistant varieties of plants. 2) Exchange of information and possibly materials in connection with new and improved methods and materials for plant disease control in general. 3) Provision for exchange of information regarding outstanding developments in plant pathology in various countries, particularly outbreaks of new or unusual diseases.

"The responses were favorable and in many cases enthusiastic. But the difficulties of carrying out such a plan are obvious, depending as it does on the voluntary services of pathologists who already are busy with pressing duties.

"The need is urgent; is not the United Nations the appropriate organization to meet it?"

Requests have been made for concrete suggestions, and the Committee is in process of formulating them.

The Committee has received proposals that attendance by members of the Society at international meetings be urged. The Committee took no action in the matter, despite the fact that the desirability of such attendance is recognized. The difficulty of financing long trips must be overcome before concrete proposals can be made. It does seem desirable that members of the Society attending international conferences might be designated as representatives of the Society.

E. C. STAKMAN, *Chairman*

Report of the Committee on Plant Disease Prevention, and Special Subcommittee on National Defense. The work of this Committee has been done almost entirely through the activities of its three subcommittees, each of which is transmitting its own separate report directly to the Council.

At the 1948 Annual Meeting the Council directed the President, in consultation with the chairman of this Committee, "to appoint a group of three to five members" to visit certain Federal Administrative Units and present to them the need for more effective protection of major crops and trees against plant diseases, with particular reference to national defense. This group, duly appointed by President Valleau, met in Washington, D. C., on February 22 and drew up a statement of principles and recommendations relative to the importance and necessity of plant disease prevention in relation to national defense. It instructed the chairman to make arrangements for presenting them to the proper authorities.

The chairman participated in two conferences with representatives of the National Military Establishment and the U. S. Department of Agriculture, at which he was given an opportunity to present the recommendations of our Committee relative to the problems of plant disease prevention in the program of national defense. Three other well-informed members of The American Phytopathological Society took part in these conferences as representatives of other interested agencies. Because of the confidential nature of these conferences, it is not possible to deal with specific recommendations in this report. Suffice it to say that responsible authorities in the military establishment and the U. S. Department of Agriculture have been made aware of the importance of plant pathology in a program of national defense. Very definite recommendations for a program of action were agreed upon. Although our recommendations have not yet been implemented, the chairman of your Committee feels that we have accomplished one of our primary objectives, namely, to place the facts before those responsible for national defense. We believe that those charged with organizing a program of national defense have been made aware of the importance of plant pathology and of the specific steps that should be taken. Much remains to be done, but the proper contacts have been made and your committee will continue to do everything in its power to see that our final objectives are reached.

J. G. LEACH, *Chairman*

Report of Subcommittee on Regulatory Work and Foreign Plant Diseases. The Committee held two meetings during the year and made a special study of the present status of the potato wart disease in Pennsylvania, Maryland, and West Virginia, at the request of the Bureau of Entomology and Plant Quarantine. Two members of the Committee made personal inspection of the eradication program in Pennsylvania which is proceeding in an orderly manner and should be completed in 1958. The situation in Maryland appears to be satisfactory with the probability that eradication will be realized in 1950. Recommendations were made to the Bureau of Entomology and Plant Quarantine that supplemental aid be given the program in West Virginia.

Resolutions passed by the Association of Land Grant Colleges and Universities at their 1948 meeting give strong support to the need for more adequate protection against the introduction of foreign plant diseases into the United States. Action on this resolution is developing through the implementation of Public Law 402.

C. R. ORTON, *Chairman*

Report of Subcommittee on Seed and Plant Material Certification. The occurrence of new plant diseases or outbreaks of previously serious well-known diseases serves well to emphasize the need for further effort toward plant disease reduction by better certification procedure for disease-free plant material. This Committee wishes to go on record in favor of increasing requisites where such will serve a good purpose, especially for materials of foreign origin.

No attempt is made here to summarize requirements already in effect or to make detailed suggestions for standards. Certain specific recommendations, however, can be

made. (1) *Seeds.* It is realized that the disease tolerance requirements for dry seed certification will necessarily vary with geographic sections, depending on the diseases which flourish in that section. This, in some cases, has been taken care of within States, but there is a need for more uniform requirements, especially for seed moved interstate. It is further recommended that seed treatment be made one of the requisites of seed certification for all seeds of plants affected by seed-borne diseases where satisfactory seed treatment methods are known. (2) *Vegetative Propagation Material* (tubers, corms, bulbs, roots, plants, cuttings, scions, etc.)—*Domestic.* As poor as it is, yet as good as it is, the certified seed program for potatoes stands as an example of what can be done in the way of cleaning up seed stocks, and setting up provisions for handling and further improvement. The potato improvement program has reached the point where most States are participating and many are cooperating on uniform standard requisites. There is need for similar programs for a large number of food and ornamental plants.

Plants noteworthy for carrying viruses in propagating material include lilies and bulb crops, gladiolus, ranunculus, strawberries, small fruits, tree fruits, and many ornamental plants. Examples of serious fungus, bacterial, and nematode diseases carried in vegetative propagation material which may be cited are fusaria attacking potato, the corm rots of gladiolus, ring rot of potato, golden nematode and rot nematode of potato, verticillium of peppermint, red stele of strawberry, and many others. Some States have made small beginnings, particularly on strawberries and tree fruits, but need more support and need to coordinate their attempts with neighbor States. All attempts should be accompanied by comprehensive research and educational programs. Regulatory provisions should be avoided in favor of voluntary procedure until stocks and procedures are established.

L. C. COCHRAN, *Chairman*

Report of the Subcommittee on Utilization of Plant Pathologists and Facilities in National Emergencies. The Committee, through correspondence among its members, has achieved the following accomplishments: 1. Prepared eight job descriptions for the utilization of plant pathologists and mycologists in the military service (copies are displayed in the Committee's folder exhibit). 2. Requested the Secretary of Defense to call a joint conference of plant pathologists and appropriate military personnel for the purpose of studying the need for and services by plant pathologists in a national emergency (copy of letter displayed in Committee's folder exhibit along with copy of Secretary Johnson's reply). 3. Considered further some of the projects that the Committee has initiated.

The Committee recommends that The American Phytopathological Society: 1. Authorize this Committee to inform members of the Society who are reserve officers about the opportunities for assignment to Research and Development Units of the Organized Reserve. 2. Authorize this Committee to cooperate with Research and Development Units of Reserve Officers throughout the nation on matters pertaining to the utilization of plant pathologists in national emergencies. 3. Continue this Committee for 1950.

The Committee informs the Society: 1. That the Military Establishment has authorized the organization of Research and Development Units of Reserve Officers at certain universities and industrial centers throughout the nation. As of August 20, the army had 85 units consisting of 2,704 officers and the navy had approximately the same number of units and officers, according to Dr. P. D. Gard, Department of Army. 2. That the Fifth Army called a conference for August 27–28 at Ft. Sheridan, Illinois, for scientists assigned to the Research and Development Units within the Fifth Army. Of the some 250 reserve officers present, six were plant pathologists.

H. H. THORNBERRY, *Chairman*

Report of the Committee on Resolutions. The American Phytopathological Society expresses its real appreciation:

To E. G. Rex, Chairman, and L. M. Black, O. N. Liming, and P. P. Pirone, members of the Committee on local arrangements, for their exacting efforts in assuring the success of the forty-first annual meeting;

To George L. McNew, Chairman, and members of the Committee on Public Relations for the enterprise and ingenuity in obtaining maximum press and radio coverage for the program of the Society;

To the American Association for the Advancement of Science for cooperation in arrangements for meetings;

To the managements and staffs of the Hotels Martinique and McAlpin for their considerate and courteous treatment of the Society and its members;

To the following sponsors whose assistance contributed so much to the excellence of entertainment and the annual dinner: Accurate Tool Co.; F. A. Bartlett Tree Expert Co.; Bobbink and Atkins; Carbide and Carbon Chemical Corp.; Davey Tree Expert Co.; The Dow Chemical Co.; E. I. DuPont de Nemours & Co.; Grasselli Chemicals Dept.; Peter

Henderson & Co.; Innis, Speiden & Co.; Lovett's Nursery; Phelps Dodge Refining Corp.; Pittsburgh Agricultural Chemical Co.; Princeton Nurseries; Rohm & Haas Co., Agricultural & Sanitary Chemicals Dept.; Somerset Rose Nursery; Stauffer Chemical Co.; Stumpp & Walter Co.;

To the Department of Floriculture, Rutgers University, for flowers at the banquet; and

To the members of the New York Convention and Visitors Bureau for efficient assistance in providing for accommodations.

The members of the Society express their thanks to President Valleau, Vice-President Tucker, Secretary May, Treasurer Richards, and Editor Helen Hart for their efforts during the year on behalf of the Society.

M. A. MCKENZIE, *Chairman*
O. C. BOYD

Elections and Appointments. A committee of the Council opened and counted the ballots for President, Vice-President, and Councilors-at-large. C. M. Tucker, J. G. Horsfall, and George L. McNew were elected, respectively, to these offices. A total of 684 votes was cast for the three presidential candidates.

The Council recommended and the Society approved the appointment of M. W. Gardner as Editor for a 3-year term through 1952, and Thor Kommedahl, J. G. Leach, John Stevenson, and Coyt Wilson as Associate Editors for the same period.

The Council and the Society express their appreciation of the editorial work done for the Society by the following whose terms expired December 31, 1949: Editor H. A. Rodenhiser; Associate Editors G. M. Armstrong, F. L. Drayton, Byron R. Houston, and M. F. Kernkamp.

L. J. Alexander was reappointed Advertising Manager of PHYTOPATHOLOGY for 1950; L. C. Knorr and L. J. Tyler were reappointed as Editor and Business Manager, respectively, of PHYTOPATHOLOGICAL CLASSICS for 1950; J. C. Walker was appointed to represent the Society as Director on the Governing Board, American Institute of Biological Sciences, for a 4-year term through 1954, and as representative to the Division of Biology and Agriculture, National Research Council, during 1950; H. C. Young, Sr., was appointed as representative on the AAAS Council for 1950–1951; Charles Chupp was continued as representative on the Board of Editors, American Journal of Botany, for 1950; H. H. McKinney was retained as representative to the American Type Culture Collection; H. H. Thornberry was continued as Society member of the Scientific Liaison and Advisory Board, Quartermaster Food and Container Institute for the Armed Forces.

Reports of Officers, Representatives, and Standing Committees as published in this report, and reports of Special and Temporary Committees (not published) were approved by the Council and accepted by the Society.

Interim Appointments. The President appointed Dr. L. M. Hutchins to represent the Society at the United Nations Scientific Conference on the Conservation and Utilization of Resources, August, 1949.

In accordance with a resolution passed by the Plant Disease Prevention Committee at the Pittsburgh meeting, the President appointed an ad hoc committee to present to the Chief of the Bureau of Entomology and Plant Quarantine, the Chief of the Bureau of Plant Industry, Soils, and Agricultural Engineering, The Secretary of Agriculture, The Secretary of National Defense, and the Director of the Bureau of the Budget, the urgent need for more effective protection of the major crops and trees against plant disease, particularly plant diseases not now present in the United States. The Committee consisted of the following members: J. G. Leach, Chm.; R. P. White, J. G. Horsfall, C. R. Orton, and G. C. Kent. See report of standing committee on plant disease prevention.

The following actions were approved by the Society:

1. J. A. Stevenson was selected as Society representative to the section on Nomenclature of the Seventh International Botanical Congress, Stockholm, 1950.
2. The officers were empowered, upon request of the Seventh International Botanical Congress, to designate representatives or otherwise accredit delegates to the Conference.
3. Payment was authorized of $900 to the American Institute of Biological Sciences for their fiscal year 1950.
4. The cost of subscriptions was set at $8.00 per year beginning January 1, 1951.
5. Continuation of publication of the PHYTOPATH NEWS for the fiscal year 1951 was authorized and $300 was allotted for this purpose.
6. Following previous custom, it was ordered that the finances of the Society and of PHYTOPATHOLOGY be audited by a certified public accountant before the new Treasurer and Business Manager, A. E. Dimond, assumes his position.

7. The Treasurer and Business Manager is authorized to incur expenses for a bond of not more than $2,000.

8. The program of the National Society for Medical Research is endorsed and the Society accepts the invitation of the Society for Medical Research to become a member of that organization, but without contribution of funds.

9. Publication in Plant Disease Reporter was authorized of the results of the poll taken by the committee on Virus Nomenclature. The President was authorized to appoint a delegate to present the results of the poll and to represent the Society at sessions on virus nomenclature at the Seventh International Botanical Congress at Stockholm.

10. The title of the Committee on Investments was changed to Finances and Investments. The committee was requested to investigate the investment policies of the Society and to make a report at the next annual meeting.

11. The following subcommittees of the Fungicide Committee were discontinued: Seed Treatments; Dusts and Sprays; Annual Summary of Results of Newer Fungicides. The Society expresses appreciation for the timely information collected and made available by the chairman and members of these subcommittees.

12. A new subcommittee of the Fungicide Committee was formed to continue in part the functions of the several subcommittees that were discontinued. This subcommittee, Testing and Results of Newer Fungicides, is authorized to consider means of publishing a combined report of the Committee on Dusts and Sprays and the Committee on Annual Summary of Results of Newer Fungicides. The committee is instructed to suggest a method of publication, estimate the cost, and inform the President, who will then poll the Council to determine the action to be taken. If expenditures for publication are authorized, income from Sustaining Associate Members shall be used.

13. A special committee to be known as the Committee on Spray Tolerances was formed. This ad hoc committee was instructed to compile information on the essential need for the use of fungicides in crop production and to present the information at the forthcoming hearings on Pesticide Tolerances called by the Pure Food and Drug Administration. The information should include a list of diseases that require chemical control and a list of chemicals that have found approval in one or more States. The Committee is instructed to apprise agricultural experiment station directors and similar officials of the importance of compiling and presenting evidence on the necessity of fungicides for profitable crop production as their responsibility to the growers. One or more members of the Committee are authorized to represent the Society at the hearings.

The following resolution was passed:

In view of the professional experience and a great weight of valid data, BE IT RESOLVED that The American Phytopathological Society endorse the proper use of chemicals as fungicides for food production.

14. The Society approved changes in the Constitution of the American Institute of Biological Sciences as communicated to the Secretary under date of July 22, 1949.

15. The Society approved the nomination of the Society of General Physiologists as a member of the American Institute of Biological Sciences.

16. The Society recommended that the Constitution be amended to provide for the election of the President one year in advance of his taking office and that he serve on the Council during his term as President-elect. The proposed amendment will be published in a later issue of PHYTOPATHOLOGY.

17. The Society gave final approval to an amendment to Article II of the Constitution, creating a new class of members to be known as Emeritus Members.

Article II, Membership, is changed to read as follows:

Section I. The Society shall consist of Charter Members, Annual Members, Patrons, Sustaining Associate Members, and Emeritus Members.

Section II. (a, b, c, d, not changed.) e. Members of 20 years' standing may become Emeritus Members upon application to the Secretary. An Emeritus Member shall retain all rights and privileges of the Society and is exempt from payment of dues, but shall not receive PHYTOPATHOLOGY unless he so desires, in which case he shall receive it at cost.

CURTIS MAY, *Secretary*

MINUTES OF THE NORTHEASTERN DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, DECEMBER 28–30, 1949

The fourth annual meeting of the Northeastern Division was held jointly with the parent society. President D. H. Palmiter presided. The Secretary-Treasurer read the minutes of the business meetings held December 6, 1948, and September 8, 1949, an account of the 1949 summer field trip in New Jersey, and the Treasurer's report of December 28, 1949, all of which were accepted. The Nominating Committee proposed the following candidates for office: President, E. M. Stoddard; Vice-President, E. G. Rex; Secretary-Treasurer, S. G. Younkin; Councilor, M. T. Hilborn. No further nominations coming from the floor, it was moved, seconded, and carried that the Secretary record a unanimous ballot for the candidates. Arrangements for a summer meeting and an autumn or winter meeting were entrusted to the Executive Committee. Resolutions were adopted expressing the appreciation of the Division for the work of the officers and local committee of the parent society in arranging the joint annual meeting and for the efforts of the local committee which carried through the successful 1949 summer tour of New Jersey.

It was unanimously agreed that "In view of the high esteem we have for our late colleague, Dr. Raymond B. Wilcox, BE IT RESOLVED that a letter be written by our Secretary to Mrs. Wilcox expressing our sympathy."

L. M. BLACK, *Secretary-Treasurer*